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Vol. LVI. *April, 1929* *No. 4*

BIOLOGICAL BULLETIN

THE RÔLE OF THE FIN RAYS IN THE REGENERATION IN THE TAIL-FINS OF FISHES.[1]

(IN *FUNDULUS* AND GOLDFISH.)

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The experiments which form the basis of this paper were planned for two purposes:

1. To reëxamine the facts, as set down by Morgan:

(*a*) The rate of growth is greatest where most material is needed to complete the typical form of the tail (1902) (*i.e.*, the controlling factors are not those usually considered as physiological ones—the availability of food or blood at the level of the cut —but certain formative factors).

(*b*) These formative factors are internal and may be expressed in terms of tension or pressure relations that initiate growth, govern the return to form, and cause a cessation of growth (1906).

2. To present, if possible, new data that would throw additional light upon the problem of regeneration and morphogenesis in the tail-fins of fishes.

Under the direction of Professor J. Walter Wilson, the experiments discussed in this paper were begun at the Arnold Biological Laboratory of Brown University (1927), and continued at the Marine Biological Laboratory at Woods Hole, Massachusetts (1928).

[1] I am particularly indebted to Professor Wilson for his valuable assistance in the preparation of this paper.

[2] Biological Laboratory, Morehouse College. (On leave 1927–28.)

Historical.

Broussonet (1786) recorded the following results of his experiments in regeneration: (1) Fins reproduce themselves in slow degrees; more rapidly in young fishes than in old; more rapidly in some species than in others. (2) "Poissons dore's de la Chine" regenerate fins: new rays can be distinguished after three months; a severed "right fin" reaches original growth in eight months; both ventral and caudal fins regenerate after oblique and transverse cuts. (3) There is correlation between liability to injury and power to regenerate (Bonnet's conception). (4) A part of the "osselets" is necessary for regeneration; otherwise new fins are not produced.

Unaware of Broussonet's work, Mazza (1890) found that regeneration takes place in the tail of the goldfish (*Carassius auratus*).

Weissman (1892) was aware of the fact that the Salamander woud regenerate a lost limb, but did not believe that fins of fishes would regenerate.

Unaware of the works of Broussonet and Mazza, Nussbaum and Sidoriak (1900) published results of experiments in regeneration on a young brook trout (*Salmo fario*), discussing, for the first time, the histology of regeneration in tail fins.

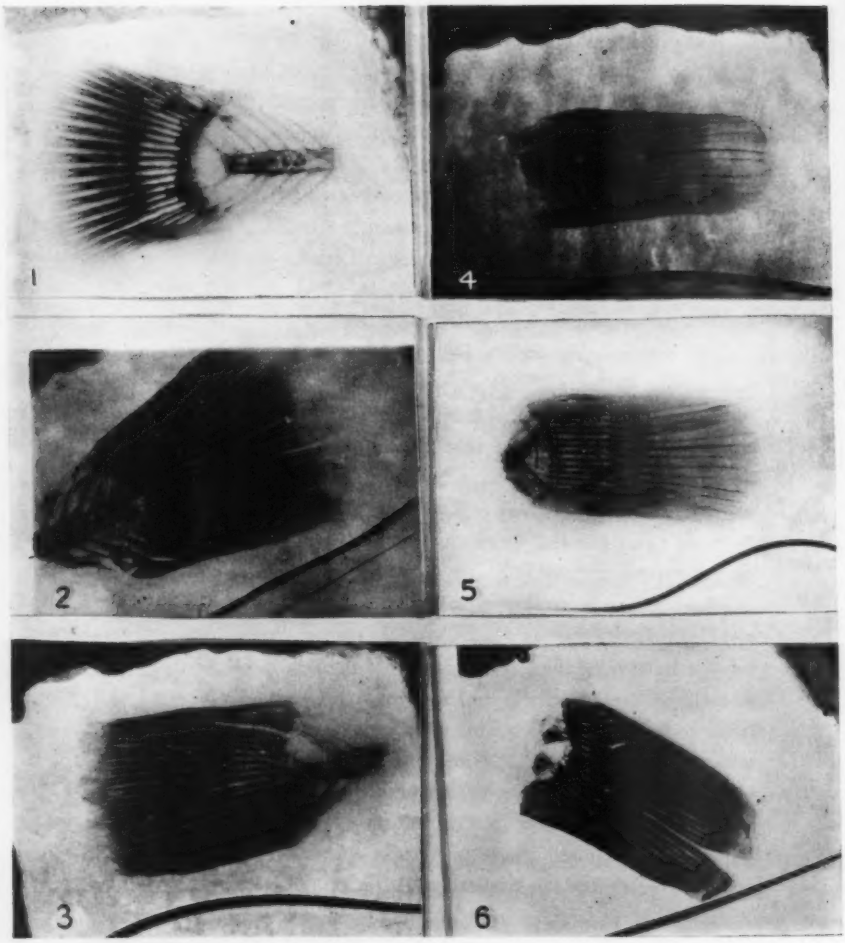
T. H. Morgan (1900) was probably the first one to experiment on the tails of fishes from a standpoint of *morphogenesis*. His work was done largely on *Stenopus, Fundulus,* and *Carassius;* his findings were published in 1900, 1902, and 1906. He held the view that *differentiation* is an expression of certain pressure relations: Typical form is established and growth is completed when resulting pressures no longer act as a stimulus on the growing region; the formed part exerts upon the unformed parts an influence of pressure. Quoting Morgan, "The failure of the maximum potential of growth over the more distal parts of an oblique surface is due directly to the new growth below it not having reached the same level, and owing to this difference there arises a pull or tension on the part that retards its maximum possible rate."

Material and Methods.

The nearness of Brown University to the collecting grounds of Narragansett Bay makes it possible to experiment upon *Fundulus heteroclitus* and to keep salt water aquaria with daily changes of water brought in by boats from the collecting grounds. The fresh water forms were in an aquarium that had a daily change of fresh water. Both types of aquaria were aereated by means of an electrically driven air pump and green water plants. Worms, wafers and granulated fish food were the types of food used. The temperature of the water was from 19° to 21° C. At Woods Hole, aquaria with running salt water were used.

Cuts were made with scissors and scapels with reference to base of the scales. (The base of the scales as shown in Text fig. *b*. is important, because of the difference of rate of growth from cuts made at each side of this circular base of scales. A strong *Sodium Chloride* solution was used for the removal of any fungus growth. Mild cases thus treated were cured, but the more pronounced cases resulted in death, though, perhaps, slightly retarded by the treatment.

Observations were largely made on living specimens wrapped in wet cloths under the binocular dissecting microscope. Some observations have been made on individuals preserved in formalin and under low power of the microscope. *Camera lucida* has been employed for some detailed observations on regeneration from squares cut in the tails of *Fundulus* and *Carassius*. Photographs of goldfish were made from chloretoned specimens while the tail was spread out in a thin film of water. The photographs of *Fundulus* were made from preserved specimens. The flesh and scales were scraped off and the skeleton of the tail was stained in *alizarin*. They were cleared and photographed in *oil of wintergreen*. The tails were cut from the body by an oblique cut which had the short end of the cut surface of the vertebral column ventral, and the extended end dorsal. This served to orient the tail readily.



FIGS. 1–6. The shapes or the forms of the tails are correlated with the mode of branching of the fin rays.

FIG. 4. The regenerate from the anterior face of a square hole grew past the posterior face of the hole from which no regeneration had started.

Experimental Section.

I.

Description of Tails Used.

The tails of *Fundulus heteroclitus* vary in shape and size (Figs. 1–6). There is a close correlation between the shape of the tail and the mode of branching of the fin rays. The more rounded the tail, the more branched are the fin rays of the tail, especially the rays bordering the dorsal and ventral regions of the tail. The rays articulate with a basal plate at the end of the vertebral column. They are smallest distally and all the rays appear the same size at their distal ends. At the proximal ends the rays are larger than they are at the distal end, and have an articulating knob or enlargement. From the knob the ray becomes smaller until it reaches the base of the scales in the tail; from the *scaly base* it becomes segmented, and more distally there is a doubling of the segments prior to the branching of the rays. This doubling of the segments increases the cross-sectional area of the rays in the middle third of the tail. There is a difference in the number of branching rays in the dorsal and ventral regions of the tail and there is also a difference in position at which the rays of the dorsal and ventral regions branch. The fin rays of the ventral region of the tail branch nearer the scaly base than those of the dorsal region, but more rays of the dorsal region branch than of the ventral region. This difference in mode of branching is already apparent in the early larval stages of the animal, being observed on about the second day after hatching.

In the diagrammatic plate (Fig. 7) it is noticed that the eleventh fin ray from the central one in the dorsal region of the tail doubles its segments and forms secondary branches. The eleventh ray from the central one in the ventral region of the tail does not double and does not form secondary branches. It is readily seen that in this tail more end surface would be exposed in the dorsal region of the tail than in the ventral region, although the cut is supposed to be at the same level in both regions. Neglect of this difference in distribution of fin-ray material gives rise to misleading suggestions as to the controlling factors in growth in tails of fishes,

when distance from base of tail is alone taken as a criterion of level without taking into account these internal structures (see page 252).

The tail of the goldfish differs from that of *Fundulus* in that the largest rays are in the dorsal and ventral lobes and the smallest ones are in the central region (Fig. 8). The rays branch only

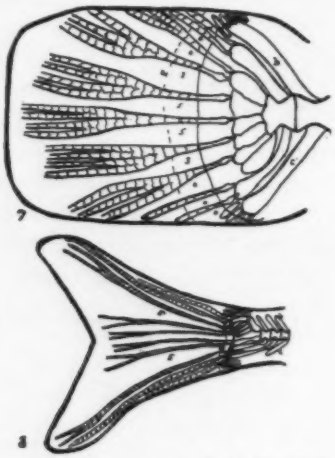


FIG. 7. Diagrammatic and exaggerated sketch of the tail of *Fundulus* which was made from the photograph in Fig. 2. The numbers indicate the omitted rays between any two. (*a*) Scaly base of the tail; (*b*) neural spines; (*c*) hæmal spines.

FIG. 8. Goldfish. (*a*) Urostyle; (*b*) neural spine; (*c*) hæmal spine; (*d*) scaly base.

once instead of twice as in *Fundulus*. Sometimes a goldfish has rays that branch a second time and sometimes in *Fundulus* the rays branch a third time. The shape of the tail is correlated with the mode of branching of the fin rays. The base of the bilobed tail of the goldfish is *heterocercal*, but the tail is *homocercal*. *A. urostyle* projects into the dorsal lobe of the tail. The rays are small near the base of the tail. Prior to branching, the rays enlarge and the segments of the rays divide to form secondary segments. This enlargement of the rays is intimately correlated with the branching of the rays, and occurs in the middle third of the tail. After branching, the rays become small distally. The final

size of all the rays is apparently equal. The rays of the lobes branch after those in the central region of the tail.

The fan-tailed goldfish has the equivalent of two bilobed tails, with the outer rays of the two dorsal lobes being attached along their long axes.

A study of the development of *Fundulus* shows that there is a *primitive natatory fold* and the mesenchymal mass from which the fin rays and the articulating base develop migrates between the extremity of the fold and the end of the notochord and the spinal cord, and from the mesenchymal mass the central rays are the first ones to differentiate. At this time the natatory fold is

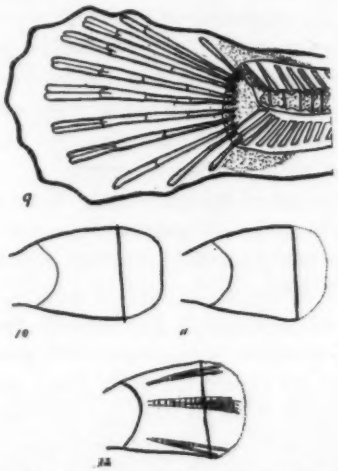


FIG. 9. A camera drawing of the tail at the stage of development when the differences in the distribution of the fin-ray material are first observed. The rays are longer and segmentation is farther from the base in the dorsal region of the tail. The rays in the dorsal region are slightly larger than those of the ventral region. One more ray shows doubling for branching in the dorsal region than in the ventral region.

FIGS. 10, 11, 12. This cut was made at the level where there is a transition from large branching rays.

farther from the base of the notochord and the spinal cord at the central region than it is in the dorsal and ventral regions. In individuals that hatched in sixteen days this streaking of the rays begins on the seventh day. The additional rays are added dorsally

and ventrally, but the blood vessels that come to pass between the rays loop in their paths before the rays are stainable vitally with *Nile blue sulphate* or with *alizarin* after fixation. On the eighteenth day (Fig. 9), or two days after hatching, the rays in the dorsal region of the tail become larger and more branched than the rays of the ventral region.

General Features of Regeneration of Tails.

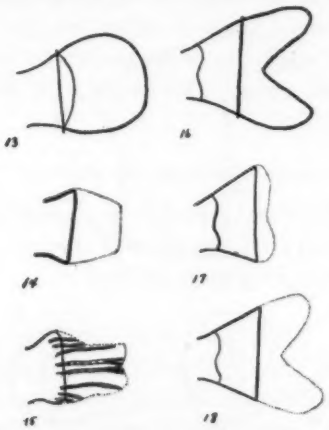
Cut surfaces of various kinds have certain features in common when they regenerate. The proliferation of new tissue, in the forms studied, has proceeded until it is visible at the end of one week in all cases. Cuts at corresponding levels in two different kinds of individuals regenerate at rates that are very close to one another. The rays appear in the new tissue in a period that varies from two to three weeks. The rays can be observed grossly by transmitted light earlier than they can be observed microscopically. This appears to be due to the particular type of condensation in the regenerate before pigmentation begins.

The form of the end of the tail is early observed in all types of regeneration, such as the *diphycercal, homocercal* or fan-tailed fish. The nearer the distal end of the tail that the cut is made, the earlier is the difference in rates of growth observed in the various regions which establish the form of the particular tail. In *Fundulus,* a *primitive diphycercal-tailed* genus, there is an early rounding of the regenerate at its distal extremity. In the *homocercal* tails of the goldfish there is a very early lobing of the tails from cuts near the distal end of the original tail. Pigmentation in the regenerate occurs in most cases after a streaking of the rays is visible. In *Fundulus,* the invasion of the connective tissue regions by pigment is useful in observing the appearance of the rays. In goldfish the gold pigment does not become visible in the regenerate until after the melanophores appear, provided that *melanophores* appear at all. The new tissue can still be distinguished from the old after six months have elapsed.

Regeneration from Cross-Cut Surfaces in the Tails of Fishes.

When *cross-cuts* are made at various levels of the tail (Figs. 10–15), the proliferation of new tissue becomes visible in about

four days. It is apparently equal in amount across the entire surface at the end of the first week. For cut surfaces in the inner two thirds of the tail, measured from the circular base of scales, the apparent rates of growth at different levels at which the cuts



FIGS. 13, 14, 15. This cut was through the region of the tail anterior to the scaly base. The rays of the center branched once at their distal ends. The dorsal and the ventral rays did not branch. Five months after the cut was made, the fish was killed and the sketch made.

FIGS. 16, 17, 18. The different stages of regeneration from a cross cut in a bilobed tail are pictured.

were made are practically equal. During the first week after proliferation has become visible, a difference in the rate of growth between the center of the cut surface and the dorsal and ventral regions is noticed. In *Fundulus* the tail is rounded up so that the typical curved form of the tail extremity is approached. In the lobed-tail goldfish (Figs. 16–18) the typical lobing is very early approached. In the former case the growth is more rapid at the center, and in the latter it is more rapid in dorsal and ventral regions of new tissue.

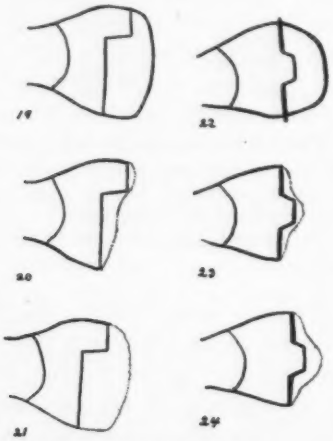
An explanation of the difference in rate in the center of the tail of *Fundulus* and the dorsal and ventral surfaces on a *cross-cut* surface may be based on the fact that more cut edge of the osseous fin rays is exposed in the center of the tail than is exposed dorsally and ventrally. This is very striking in view of the fact that in

the bilobed tail of the gold-fish the greater quantity of exposed fin-ray material is dorsal and ventral and diminishes toward the center.

The fin rays in the tail of the goldfish are so arranged that the smallest rays are in the center of the tail and the largest rays in the dorsal and ventral lobes of the tail, so that in the *cross-cut* more end surface of fin ray is exposed in the dorsal and ventral regions than in the center. This accounts for the early lobing of the tail.

Regeneration from Angular and Partial Cut Surfaces.

The regeneration from a *partial cut* or *angular cut* in general is not very different from that of other types of cuts, except that a more limited area of the tail is involved.



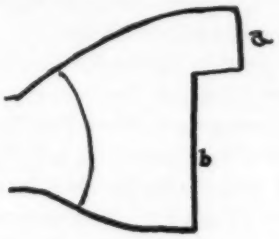
Text Fig. *e*. Regeneration from partial surfaces in *Fundulus*.

Figs. 19, 20, 21. Regeneration from a small dorsal and distal partial surface is slower than that from the central proximal cut.

Figs. 22, 23, 24. The retardation in regeneration from a central distal and partial surface is not as great as that from a dorsal distal and partial surface.

In *Fundulus* cut so that the distal part of the partial surface (Figs. 19–21) (*a*) (Text Diagram *a*) is dorsal to and smaller than the proximal ventral surface (*b*), growth is more rapid from the latter surface (*b*) than from the former surface (*a*), and the

most rapid growth on the proximal ventral cut is in the region of the central rays of the tail. The proximal surface regenerated faster than the distal surface, and when the rounded distal end of the tail was formed, they proceeded together to complete the parts of the tail then lacking.



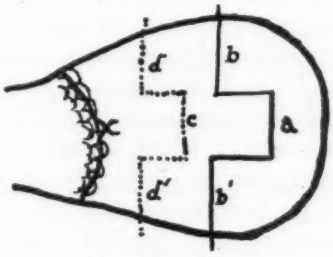
TEXT DIAGRAM *a*.

Quoting Morgan (1906), from page 482:

"The rate of regeneration from the outer *partial cut* surface is greater the broader, *i.e.*, the higher dorso-ventrally, the cut surface. This result shows that the retardation is directly connected with the height of the cut surface, and only secondarily with its distance from the base of the tail."

In other words, Morgan finds it necessary to conclude that tall *partial cuts* are less influenced by pressures and tensions than small ones. But it seems more likely that the regeneration is faster from the tall *partial cuts* because more rays are cut and more surface is exposed than in a smaller cut.

When there are three approximately equal partial cuts (Text Diagram *b*) with the portion including the central rays distal, and



TEXT DIAGRAM *b*.

the ventral and dorsal portions proximal, two types of regeneration are obtained.

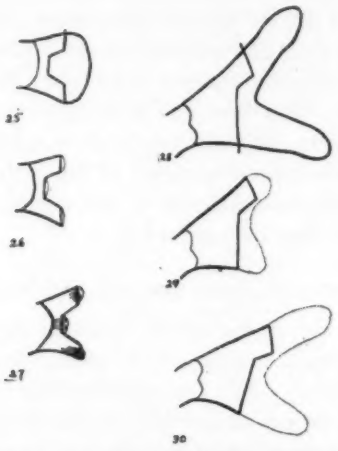
When the *partial cuts* are made so that the distal surface (*a*) is through the tertiary branches of the central rays and the proximal surfaces (*b* and *b'*) are through secondary branches of rays that are adjacent to the central ones, the rate of growth is more rapid from the proximal surfaces (*b* and *b'*). In such a case, the rate is slowest from the rays that border the dorsal and ventral surfaces. When the cut is made so that the partial distal surface (*c*) passes through the central rays before they branch and the proximal surfaces (*d* and *d'*) are nearer the scaly base of the tail (*x*), then regeneration is faster from the distal surface (*c*) than from the proximal surfaces (*d* and *d'*). In neither case is the distal central surface retarded in its rate of growth until the proximal surfaces have regenerated enough material to form a rounded distal end.

The results from the three equal *partial cuts* seem to be due to the length of the distal partial surface; for if the distal cut surface is such that the end surface of the rays is exposed at their largest point or some point larger than the point of the dorsal or ventral rays, the distal surface will regenerate faster. But if the distal edge is at a point where the rays are smaller and less surface is exposed than in the proximal regions, then the rates will be reversed.

When a *dorsal distal partial cut surface* is compared with a *ventral distal partial cut surface* at apparently the same level in the tail (Figs. 25–27), the rate of growth, if the level of comparison is near the scaly base of the tail, is faster in the *ventral distal partial cut surface* than it is in the *dorsal distal partial cut surface*. But if the level of comparison is in the middle third of the tail, the rate of growth is faster in the *dorsal distal partial cut surface*.

The explanation for this reversal in the rate of growth between the dorsal and ventral regions lies in the fact that the distribution of the larger amount of fin-ray substance is reversed in the two regions. The rays branch nearer the scaly base in the ventral region than in the dorsal region of the tail, but more fin rays branch in the dorsal region. Therefore, near the base of the tail the rate of regeneration is faster in the ventral region because

the cut was made through the point of doubling of segments prior to branching in the ventral region, whereas this doubling of segments prior to branching had not started in the dorsal region of



TEXT FIG. *f*. Regeneration from partial surfaces in *Fundulus* and in a bilobed goldfish.

FIGS. 25, 26, 27. The regeneration from two free partial distal surfaces in the tail of *Fundulus* shows regeneration faster dorsally than ventrally. The cuts were made as far as it appeared in the same region and level in the tail. As far as the rays are concerned, the cuts are made at two different levels, with more secondary branches cut dorsally than are cut ventrally.

FIGS. 28, 29, 30. Regeneration from a small distal and dorsal partial surface is not retarded by the more proximal surface. Regeneration from the ventral region of proximal surface is faster than that from the central region of the same surface.

the tail. But in the middle third of the tail the level of the cut passes through the point of doubling of the segments of the primary branches or through the secondary branches, whereas in the ventral region the plane passes through the point of doubling of the segments of the secondary branches or through the tertiary branches.

From a *distal dorsal partial cut* as (*a*) in Text Fig. *a*, in the tail of a goldfish (Figs. 28–30), the rate of regeneration from the distal surface is more rapid than the rate from the proximal surface. There is no "*holding back*" on the free distal partial sur-

face. From a free distal central surface as (*a*) in Text Fig. *b*, the rate of regeneration is less rapid than from the proximal partial surfaces (*b* and *b'*). In this case, the "*holding back*" or retardation of growth is on the central region of the tail.

In the bilobed tail of the goldfish, *partial cuts* regenerate at rates opposite to those of *Fundulus*, the rates being faster dorsally and ventrally where the rays are larger when the *partial cuts* in these regions are either proximal or distal to the central region. Just as in *Fundulus*, the initial rate is about equal, and if the cut passes through the branching region of the central rays, and the other cuts through the distal ends of the dorsal and ventral rays, the rate is faster from the central rays.

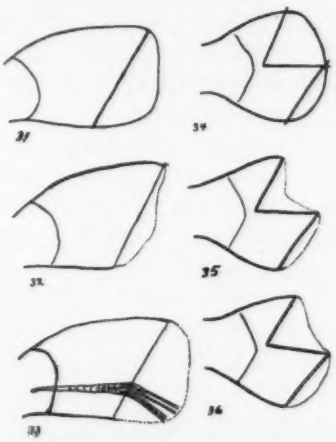
Regeneration from Oblique Cut Surfaces.

From *oblique cuts* in the tail of *Fundulus*, the rate of growth is faster in the proximal region of the cut and slower in the distal region (Figs. 31–33). The new rays from the *oblique cut* stand at an angle with the cut surface that approaches a right angle, and it is from five to six months before the necessary adjustment for straightening the rays occurs.

The visible change in the adjusting regenerate of the tail of the fish is a closer compacting of the regenerated ray with the cut edge of the old ray and an apparent increase of rigidity of new rays as they become more like the old rays. This compacting and increase of rigidity of new rays may explain the pulling of the new part into the axis of the removed part. The compacting of new rays on the ends of the old rays and the gradual becoming rigid of the new ones, exerts a leverage influence on the regenerate. The power and fulcrum are nearly at the same point, hence a rather slow pulling into line of the new part. This adjustment takes from five to six months from a marked oblique cut in *Fundulus*.

The exposure of the end substance by an *oblique cut* would explain the fact that the new tail is at an angle with the old, if it is true that the ends of the rays initiate growth in the tails of fishes. The end of the ray is cut at an angle that exposes an oblique surface from which the new tissue would grow at the rate that it would from a transverse cut across the surface at the same level.

When Morgan noticed, as a result of *partial cuts* in the tail of the same fish, that there was a more rapid rate of growth of the part nearer the base of the tail, he stated that the factors



TEXT FIG. *g*. Regeneration from oblique cuts in the tail of *Fundulus*.

FIGS. 31, 32, 33. The rate of growth from an obliquely cut surface is faster proximally than it is distally. More fin-ray end substance is exposed where the rate of growth is faster.

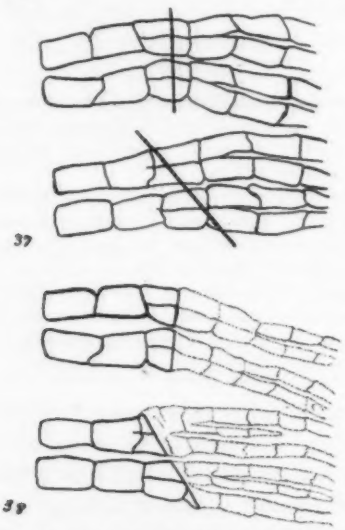
FIGS. 34, 35, 36. When two oblique cuts are so made that the distal end of dorsal surface is the same distance from the base of the tail as the proximal end of the ventral surface, the rate of growth is faster at the latter. The two points of comparison are not at the same level in terms of the amount of exposed fin-ray material. More secondary rays are cut across in the proximal region of the ventral surface. In the proximal region of the dorsal oblique surface more ray stubs are exposed than in either of the two surfaces; from there the rate of grqwth is faster.

there were different from those controlling the rate from an oblique cut surface. By means of a series of elaborate *oblique cuts* and *partial cuts*, he set out in 1902 to prove this point. In 1906 he had satisfactorily proved it, although he left an opening for modification of the theory as long as the general principles were retained.

In *Fundulus*, the slow proximal rate of growth in the dorsal or ventral *oblique cuts* can be explained by the fact that the fin rays severed by the proximal cut are first or second order rays, while the distal ones are third order rays. Also that in some

cases, the proximal end passes through the point of branching of some rays. In some cases even here, the regeneration is faster in the center of the *oblique cut*. The type of regenerate is dependent upon the points at which the cut begins and ends. Tails of *Fundulus* vary in the time of branching of the rays and and various shapes of tails are produced by regeneration which depends on the mode of branching of the fin rays.

A marked *oblique cut* of a fin ray which exposes more end cut surface than a transversely cut fin ray at the same level will regenerate more new ray stuff than the transversely cut ray. If the normal pattern of branching is for two orders of fin rays, primary and secondary ones, it is possible for the new ray material from the obliquely cut ray to differentiate into three orders of rays. Figures 37 and 38 show the regeneration from obliquely cut fin rays in the ventral lobe of a goldfish compared

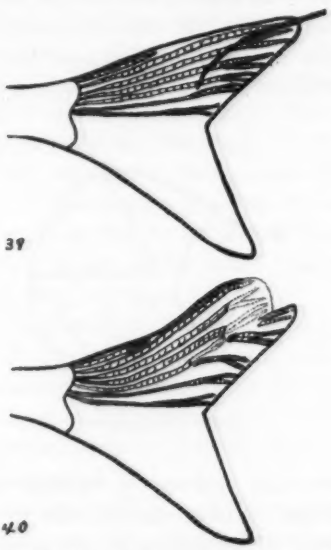


TEXT FIG. *h*. Comparison of regeneration of an oblique and a crosscut in rays.

FIGS. 37, 38. The rays in the dorsal lobe of the tail were cut transversely and those of the ventral lobe were cut obliquely. The end surface exposed by oblique cut was the greater. From the oblique surface, additional secondary rays or branches were formed in the regenerate and from the transverse surface only the normal number of rays regenerated.

with the regeneration from transversely cut rays in the dorsal lobe at the same level in the same tail.

The regenerated ray from the oblique cut may be larger than the ray which gives rise to it. This shows that the cut surface regenerates along its entire surface. More fin-ray end sub-



TEXT FIG. i. Regeneration from oblique cut in the dorsal lobe of the tail of the goldfish.

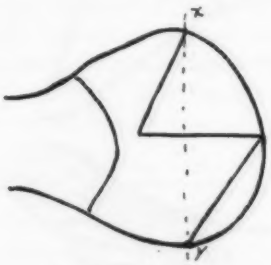
FIGS. 39, 40. Regeneration from an oblique cut in the dorsal lobe of the tail of a goldfish. An extra lobe is formed in the tail by rays that grow up and around their dissevered posterior mates.

stance was exposed. The view that the fin ray exerts the initiating influence is in harmony with the theory of Morgan in that he admits that the fin ray must be cut so that an end is exposed in order for regeneration to take place.

The shape of the tail can be altered by an *oblique cut* into the lobe of the tail when no part is removed. There is healing and also regeneration of rays from both sides of the cut. The rays from the anterior face of the cut grow upwards and around the severed posterior rays to form a new lobe in the tail (Figs. 39 and 40).

The form or shape of the tail does not appear to depend upon any pressure or tension regulating system. The fin rays determine the shape of the tail by their mode of branching and the direction of their growth.

When *double oblique cuts* are made so that the proximal level of the ventral cut is on a perpendicular axis with the distal level of the dorsal cut surface, the rate of growth is faster in the proximal region of the ventral cut surface than it is in the distal region of the dorsal cut surface (Text Diagram *c*) (Figs. 34–36).



TEXT DIAGRAM *c*.

The most convincing evidence by Morgan that the rate of growth from oblique surfaces was not due to the nearness of one part of the base of the tail, was a cut in which two oblique surfaces were made in the same tail; neither cut directly connected with the other, and the distal end of one cut at the same level as the proximal end of the other. In such a case, the regeneration from the surfaces at the same level were not so close together. His conclusion is that the proximal end exerts a retarding tension on the more distal level of an oblique cut.

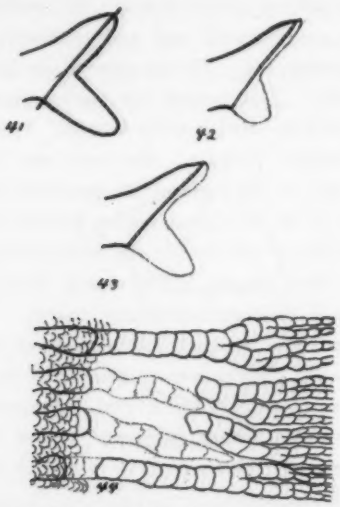
In *Fundulus,* the rays are larger in the center of the tail and by normal branching, the central rays reach the minimum size much farther from the base than do the outer fin rays. From double oblique cuts, as before-mentioned, regeneration at the same level of the tail was faster on the proximal surface of one cut than at the distal surface on the other cut. In *Fundulus,* the longitudinal cut in this experiment is adjacent to, or includes the largest rays of the tail, the central rays. The dorsal oblique sur-

face at its distal end is at the end of the final branches of the first (about five) few rays coming from the base of the tail, and at its proximal end intersects the longitudinal cut and the central rays. The ventral oblique surface has its proximal end distal to the final branches of the fin rays that leave the base of the tail. The dorsal cut passes through the smallest branches of the central rays at its distal end and increasingly larger rays towards its proximal end. Hence growth is faster proximally and slower distally. The ventral cut passes through the larger rays proximally and the smaller ones distally. The rate of growth is faster proximally. Further, the rays are correspondingly smaller in most tails at the level of the proximo distal axis (x, y —Text Diagram c) in the dorsal region than in the ventral one. The rays branch nearer the base in the ventral region than in the dorsal, but more rays branch in the dorsal than in the ventral. They tend to reach their minimum size sooner. The middle of the ventral oblique cut is really its fastest point of growth. Moreover, there is a much greater rate of growth at the proximal end of the dorsal cut than at the proximal end of the ventral cut. This is apparently due to the visible difference in size of the rays and the quantity of exposed surface. The rays are different at the two points (x, y) compared.

Assuming the *formative influence* of the rays themselves, and that quantity of cross-cut ray material determines the rate of growth, it will be seen that the same factors controlling the rate of growth from the cross surfaces in the tails of fishes govern the growth from the oblique surface.

Regeneration from an obliquely cut surface in a bilobed tail is faster in the dorsal and ventral lobes when the cut is only slightly oblique. Marked *oblique cuts* regenerate fastest at the proximal end of the cut, and slowest at the center of the cut (Figs. 41, 42, 43). At some points the rate of growth is faster at the center of the cut than at the distal end. Lobing of the tail in the oblique cut is noticed almost as early as in the case of the cross cut in the bilobed-tail fish. The first proliferation of tissue in *oblique cuts* which is observed in about a week seems equal in rate along the entire cut surface. The general tendency, however, is for the rate to be faster at the proximal end, but this is

not pronounced until after the second week. The lobing occurs much earlier when the oblique cut is nearer the original point of lobing of the tail, though when even more than half of the tail is removed, the lobing is noticed before that quantity of new tissue has been restored.



TEXT FIG. *j*. Regeneration from obliquely cut surfaces in the tail of a goldfish and regeneration in a small hole cut in the tail of *Fundulus*.

FIGS. 41, 42, 43. The regeneration from an obliquely cut surface is faster distally than it is in the more proximal central region. The rate of growth is fastest at the most proximal region. The most distal and proximal regions are regions where the rays are larger and where the cut exposes more end substance. More end substance is exposed at the proximal end than at the distal one.

FIG. 44. The new rays from the anterior face of the hole do not connect with their mates at the posterior face of the hole (*Fundulus*).

In the bilobed *Carassius* (goldfish) the rays in the center reach their minimum size first, while the dorsal and ventral rays are much larger, hence much longer. The smaller the branches of the rays are, the slower is the rate of growth in the region of their cut surfaces.

When a *double oblique cut* is made in *Carassius*, so that the distal end of one is at the level of the proximal end of the other in the bilobed tail, the rate of growth is faster at the proximal end.

In some cases, however, the cut can be made so that the distal end of the *dorsal oblique cut* regenerates at practically the same rate as the proximal end of the *ventral oblique cut*.

In the bilobed tail, the longitudinal cut is adjacent to, or includes, the smallest rays in the tail and hence the shortest ones. The distal end of the dorsal *oblique cut* is at the same level as the proximal end of the ventral oblique cut. The distal end of the ventral cut intersects the distal end of the longitudinal cut. The proximal end of the dorsal cut intersects the longitudinal cut near the point of branching of the central rays. The point of the most rapid regeneration is on a line extended directly from the outer ventral ray which is the largest ventral ray, and the most proximal point of the cut. The proximal point on the dorsal cut sometimes shows a more rapid rate of growth than the more distal points on the same surface.

The outer ray, while itself larger than any one ray, does not divide, or if it does, not as early as the rays on the inner surfaces. Cuts through a ray just at the point of division expose more surface as a result than do cuts of a single ray, and that which is not at the point of division is slightly larger than the one that is dividing. Near this is a region where the rate again is faster on the ray that has not reached its minimum in size, for the branches after separating do not expose as much surface as the two when they first separate. Whenever the proximal end of the dorsal oblique cut is distal to the point of branching of the central rays of the bilobed tail, the regeneration is faster distally than it is proximally. Morgan's theory would assume that suddenly a region of great retarding tension was reached that held back the center and the proximal end of the oblique cut. It seems, however, that the rays near the center are smaller, hence do not have to grow so far to attain minimum size.

The mode of growth is just the opposite in the bilobed goldfish type from the type of *Fundulus* and seems to be associated with the fact that the fin rays are just the opposite in size arrangement.

In the elaborate fan-tailed goldfishes the longer parts in the normal tail are supported by the larger rays. The regeneration

is faster where the cut exposed the greater amount of cut end substance. When two parts of such a tail—one directed posteriorly and one directed ventrally, are cut off, it was observed that both regenerated equally. Normally, the two parts had approximately equal lengths, and there is a very close correlation between the sizes of the rays. They regenerated equally in rate or with no noticeable difference.

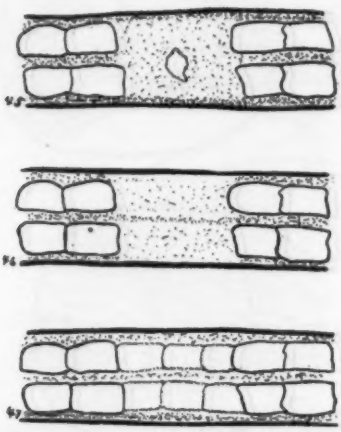
When an *oblique cut* was made of a fan-tail fish so as to remove the entire piece that is ventrally directed and the outer margin of the adjacent posteriorly directed part so as to present an unbroken cut surface, regeneration took place faster proximally than distally. Examination of the tail showed that the proximal cut passed through the main body of the branches of the first order and that the distal cut passed through the distal ends of the branches of the second order. In the fan-tailed forms as in the bilobed forms, two orders of branches is the typical condition. Hence regeneration takes place more rapidly from the cut ends of rays where more ray surface is exposed.

Regeneration in Square Cuts in the Tail Fins of Fishes.

Small squares cut in the tails of fishes provided a very suitable means of studying regeneration from four surfaces in the same tail—two longitudinal and two transverse surfaces.

Regeneration takes place only from the two transverse surfaces of the square and only healing takes place along the longitudinal surfaces. Cuts nearer the base are better in that they can be made much larger and do not break as easily as the more distal ones. In small cuts 0.2 × 0.2 cm. square, regeneration takes place by a multiplication of cells around the cut ends of the fin rays and gradually filling in along the longitudinal surfaces by migration from the cross-cut surfaces until a compact mass completely fills the cut square. From the anterior cut surface of the square and from the ends of the cut rays the new rays appear during the third week following the cut. No rays streak out from the posterior or distal face of the square. Sometimes the rays do not unite with their mates, but become distorted or continue to grow and encroach upon the connective tissue region distal to the posterior face of the square (Fig. 44).

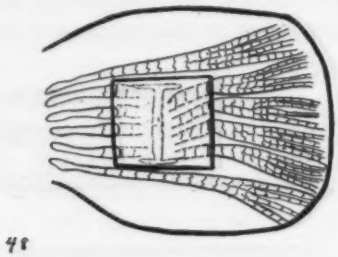
But usually the rays unite with their mates at the posterior surface and form the original missing number of segments (Figs. 45–47). Small fragments of rays broken in cutting migrate to the surface of the cut and are eliminated by rupturing the surface of the closed wound or the regenerated tissue.



TEXT FIG. *k*. Regeneration from a small hole cut in the tail of goldfish.
FIG. 45. Granulation tissue before hole is completely closed.
FIG. 46. After closure of hole.
FIG. 47. The new rays united with their mates.

From squares of 0.4 × 0.4 cms. a larger breadth and length, regeneration may occur from the posterior and anterior face of the square (Figs. 48–49). The hole never closes and from the anterior face rays continue to grow beyond the posterior face of the cut, in some cases to the original length of the tail. From the posterior face, the maximum length of the regenerated rays so far obtained in *Fundulus* has been eight segments. In several cases of large cuts no growth started from the distal face of the cut and the proximal face produced rays before reaching the distal face. In these cases the proximal growth continued beyond the distal face and did not connect with it. On the distal face there was only healing over. There was no regeneration along the longitudinal surfaces of the square (Fig. 4).

In a goldfish in which a large square had been cut and which subsequently broke, some important facts were observed. In the breaking of the square two posterior faces were left intact



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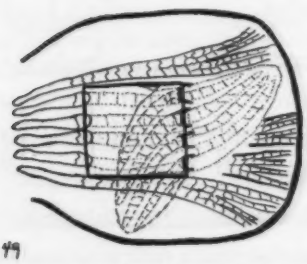

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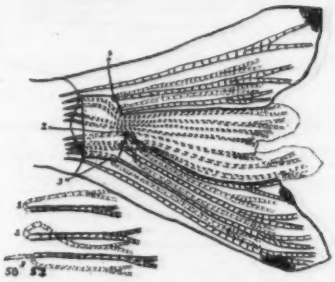
TEXT FIG. *l.* Regeneration in large holes cut in the tails of *Fundulus.* FIGS. 48, 49. The rays from the anterior and from the posterior face of the square have regenerated. The rays from one face of the square pass out to one side of the tail and those from the face pass out to the other. Eight segments have been formed in the reversed direction.

as only the center broke out (Fig. 50). Regeneration was faster from the anterior face and rays of the regenerate curved around the edges of the posterior faces from which new tissue was just proliferating. These rays continued growing to form the center of the tail and also three rays in the dorsal lobe of the tail and two in the ventral lobe. At the end of 45 days, eight segments had been formed in the reversed growth from the posterior face of the square. By 73 days after cutting, the rays from the posterior face of the square had reached the same number of branches as were in the part distal to the posterior face of the cut. A complete reproduction of the skeletal elements from the posterior face of the square was obtained, as would have been the case had a simple cross cut been made at that level.

The form of the lobing of the tail was altered in the beforementioned goldfish. The normal lobing of the tail was altered to form a lobe within a lobe, as is shown in Fig. 50. The lobe of the tail was altered by the rays from the anterior face of the square that were originally the first rays of the ventral and dorsal lobes. These rays continued in their growth until they reached their limit; their limit was beyond the point of the original center of the tail, even after curving to enter the central portion. The original central rays ceased to grow at their orig-



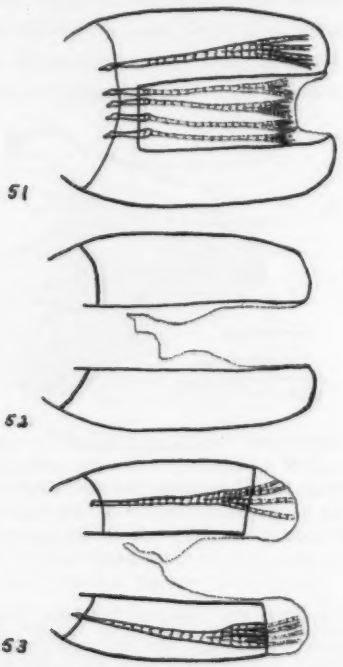
TEXT FIG. *m*. Regeneration in a large hole cut in the tail of a goldfish.

FIG. 50. A drawing of a tail with a large square that had its distal center broken out. (1 and 2) Enlargements of two rays that reversed in the opposite direction. (3) A ray growing from the anterior face of the square unites with a reversed ray from the posterior face of the square at the point of the reversal.

inal limit and a lobing was formed between them and the distorted rays. A second lobing may be said to exist between the distorted and the original lobes. Growth through the center has no retarding influence on rays that normally belong in the lobes.

Reversal of rays in their direction of growth in square holes and formation of all the branches that would have been formed if a cross cut had been made at the level indicate that the power to regenerate and differentiate rests within the fin rays themselves, for they were completely disconnected from the base of the tail. One ray from the anterior face of the hole connected with a ray with reversed growth. The ray from the anterior surface ceased growing and the one from the posterior surface which was nearly complete continued to completion; (the reversed part exits from

between the two rays at their point of fusion in a somewhat lateral direction). *Cessation of growth* in the anterior ray seems related with the fact that its surface was no longer exposed, for it had not yet reached the minimum size, and would have continued to grow if it had not met the ray from the posterior face



TEXT FIG. 11. Regeneration after picking out the rays in a given region of the tail in *Fundulus*.

(Comparisons were made after four and one-half weeks.)

FIG. 51. Four central stubs were left in the tail. The new rays branched and the regenerate filled out space made by the cut.

FIG. 52. The entire four central rays were removed and only unformed connective tissue returned. Alizarin staining did not show any rays. The base of the tail was not injured.

FIG. 53. The entire four central rays were removed. Staining showed small rays coming out from the articulating basal plate. There was no form in the regenerate. The base was broken in removing the rays. From cross cuts in the remaining portion of the tail, rays regenerated in three weeks and were visible without staining.

of the hole. It is not obvious how Morgan's pressure theory would explain these phenomena.

Regeneration after Removal of the Fin Rays.

Regeneration of the tail when some of the fin rays are removed has been obtained (Figs. 51–53). The rays are picked from the tail in a given region with forceps and a hole is thus made in the tail, as the adjoining tissue is also removed except in the muscle region of the tail. Healing of the wound may take place. If the rays in a goldfish are removed so that a central small ray is left adjacent to a long ray of the lobe, healing may take place so that the quantity of tissue added is the same as that between any two normal rays.

If a very large area from the tail is removed by picking the rays out from the base, a permanent separation in the lobe persists until new tissue grows out from the base. If a transverse cut is made in the portion of the tail in which the rays are intact at the same time that the rays are picked out, the rate of growth and establishment of typical form is faster by weeks than the rate of growth and establishment of form in the region from which the rays were removed. It does appear, however, that it is possible for new rays to grow out from the base when the old ones have been removed. This has been true in three fish—one *Fundulus* and two goldfish. Many fish, however, have not regenerated rays. It is difficult to make sure in such an operation whether a piece of ray has been left or whether the base of the tail has been injured, either of which conditions may account for new rays being produced. It appears from the stained and cleared tail that the new rays replacing those completely picked out in the tail of *Fundulus* came from the articulating base which was injured in the removal of the old rays.

Discussion.

Broussonet first showed that if the dorsal fin was cut off so that none of the "*osselets*" is left, wound closure takes place by a closing of the cut surface by the *ectoderm*, but no regeneration occurs. Morgan and others have repeated the experiment with the same results. The tail-fin will not regenerate if all the fin

rays are cut off by means of a cut anterior to their articulation with the neural and hæmal spines of the vertebral column.

When a ray is split by means of a longitudinal cut it repairs itself, but there is no lateral regeneration of the ray substance (Morgan). The surrounding tissue is simply replaced between it and the adjacent ray. When new growth from a partial cross cut reaches a split and repaired ray which has no external adjacent mate, then and only then is there a filling-in of new material in a lateral direction along the longitudinal cut. Morgan states that the ray must be cut across if regeneration is to occur; regeneration from ventral, pectoral and dorsal fins shows that growth occurs in any direction and any plane from a cross cut. Additional evidence for regeneration from a cross cut in any direction is presented as a result of a reversal of growth from the normal to the opposite direction in the tail-fin from cross-cut faces of a large square.

Ryder (1882) in discussing the development of the tail in fishes points out that in *Alosa* and in *Pomalobus* the tail is fan-shaped before the rays are developed, whereas in *Salmo* and *Oncho-rynchus* the fan-shape is not developed until after the rays have appeared. In *Gambusia, Siphostoma* and *Hippocampus* there is no primitive natatory fold from which the tail develops.

Braus (1906) showed that in embryonic *Elasmobranchs,* the *cartilaginous fin rays* would develop independently of the muscle buds of the fin by separation and transplantation of the fin-ray bud. This was also shown to be the case in some *teleosts.*

Harrison (1925) and Detwiler (1925) have shown in *Amblystoma* that the forelimb develops from a *self-differentiating* and *equipotential* system. The *mesenchymal anlage* may be transplanted and it will differentiate into a limb without its usual nerve supply and dependent upon blood for nourishment only. Detwiler even showed that the development of the forelimb is not dependent on the shoulder girdle. They suggested that the growth of the limb may be controlled by the distance of a part from the center of the bud; after arriving at a certain distance from the center of the anlage, growth would cease. Other mesenchyme could, however, in a restricted sense, due to varying potencies, simulate the extirpated anlage and produce an ap-

parently normal limb. The explanation of these experiments is based on the *mesenchyme* as a formative factor in growth and seems to be in the same category as the explanation offered for the types of regeneration in the tails of fishes.

To Morgan, the early laying down of the distal form of the tail is due directly to differences in tensions and pressure relations between parts. In *Fundulus* there is a "*holding-back*" tension dorsally and ventrally that produces the rounded tail. In bilobed Carassius the "*holding-back*" tension is greater in the center, and the dorso-ventral surfaces grow more rapidly to form the bilobed tail. From partial cross surfaces at different levels in the same tails, Morgan observed that the rate nearer the base of the tail was slightly faster than at a more distal cut surface. Quoting Morgan: "From this evidence there does not seem to be any doubt that when two *cross-cut* surfaces are present on the same tail, the new part generally grows somewhat faster from the inner of the two surfaces. Comparing this result with the growth when the whole tail is cut off squarely, the conclusion seems highly probable that the differences in the rate of growth over the outer and inner cut surface of the same tail is due to the region of the cut, and not to a regulative influence of one region on the other. This factor may also be present in the regeneration from an oblique surface, but in addition there is also present a regulative influence that holds in check the regeneration from the more distal parts of the new tail."

Barfurth (Morgan, 1902) performed some experiments on tadpoles by means of oblique cuts, and on the regenerate from such cuts, to find the forces that bring the regenerate into the position of the removed part. He observed that from oblique cuts the regenerate makes an angle with the oblique surface that approaches a right angle. Barfurth believes that swimming caused the regenerate eventually to come in line with the old part. By tying down regenerating tadpoles, to prevent swimming, some made the adjustment, but some did not. The evidence that he presented is not conclusive.

Inasmuch as the fin rays are of a mesenchymatous origin, and because of the independence of the muscle buds of cartilaginous rays in *Elasmobranchs* and some teleosts, it seems that the possi-

bility of the fin rays themselves playing a formative rôle in growth rate and morphogenesis in the tail-fins is more than probable. An explanation on such a basis was arrived at as a natural result of an attempt to apply several formative systems and theories that have been suggested; namely, the nervous system, circulatory system, distance from the base of the tail, *axial gradient of metabolism*, and the *tension regulating* mechanism of Morgan.

When the mesenchymatous tail-fin anlage differentiates into rays and connective tissue, the contained formative influences could either be distributed into the formed parts; *e.g.*, to the rays as a result of differentiation, or remain in the tail bud in the form of undifferentiated tissue. But, since regeneration of the tails is due to growth by mitoses at the level of the cut instead of migration, it seems that the result of original embryonic differentiation must be the segregation of the formative influence to the fin rays, or to an interaction of products of differentiation at the level of the cut. Of the two alternatives, the former seems to be the more plausible. The reversal of direction of growth and branching to duplicate the distal parts of the rays from square holes seems to support such a view, for here the rays are disconnected from the base.

The evidence presented in this paper concerning the rate of growth from various types of cuts is essentially in agreement with the results of Morgan. Morgan showed that the fin rays must present an exposed transverse surface in order that regeneration may take place. He thought that the rate of growth in the different regions of the tail is due to differences in tensions and pressures in different parts of the tail, and that proximal ends of oblique cuts exert a "holding-back" tension on the more distal portion of the surface. The form of the tail in various fish is due to differences in pressures and tensions. Evidence has here been presented to show that the fin rays play a formative rôle in the regeneration of the tails of fishes. The mode of branching of the fin rays and the form of the tail are intimately correlated, and regeneration has been shown to take place faster from surfaces where more fin-ray material has been cut across. Braus has shown that the fin-ray anlage will differentiate independently of the muscle bud of the tail, and other mesenchymal

structures have been shown to differentiate independently of the muscular and nervous systems. It is therefore concluded that in the development of the tail of the fish, the rate of growth of the fin comes to be regulated by the size of the rays, and in regeneration the rate of growth and consequently the form is controlled locally by the cross-sectional area of the fin rays exposed.

Summary.

1. Regeneration is faster from the level of the cut that exposes more fin-ray cross surface. The difference in arrangement of the fin rays in the tails of *Fundulus* and goldfish accounts for the rate of growth being faster or slower at opposite regions of the two types of tails.

2. Regeneration from square holes cut in the tails of fishes shows that a reversal of growth of the rays from the posterior face of the hole is possible. Healing in the longitudinal faces and repairing of the injured rays take place. Regeneration takes place only from the cross-cut ends of fin rays. The reversed rays are of the same branching pattern as the distal portion from which they are produced; that is, the same pattern that a cross cut would regenerate posteriorly from that level.

3. When rays that normally would grow into the lobe of the tail are displaced into the central portion of the tail of a goldfish, they are not "held back" by any tension in the center of the tail. The rays continue to grow and form abnormal lobes in the central portion of the tail.

4. Correlation between size and branching is brought out by two types of cuts. (*a*) A cut surface anterior to the scaly base of the tail in *Fundulus* regenerates "abnormal" rays. The shape of the tail can thus be changed from rounded to straight, and persists as such for more than seven months. (*b*) An oblique cut at the point of doubling of rays in the goldfish exposes more cross surface than a straight cross cut. The regenerate from such a surface has produced a third set of branches instead of two sets, as would be typical in the goldfish used.

5. There is a minimum size of all rays in a particular kind of tail, and the size is apparently equal for all the rays.

6. The evidence herein presented suggests that the fin rays are self-differentiating structures.

(*a*) The initiation of growth is due to cutting the fin rays crosswise.

(*b*) Rate of growth seems to depend on the amount of exposed surface of the fin rays.

(*c*) Cessation of growth appears to be due to the attainment of the minimum size of the ray at which no further growth takes place.

(*d*) Form of the tail appears not to be associated with pressure or tension relations but with the mode of branching of the fin rays and hence their size and the formative influences that they possess.

LITERATURE CITED.

Braus, H.[1]

'06*b* Ist die Bildung des Skeletes Ven Den Muskelanlagen Abhängig? Eine experimentelle untersuchung an der Brustflosse von Haiembryonen Morphol. Jahrb., 35. Also, Exp. Beitt. Z. Morphol., 1.

Broussonet[1]

1786 Observations sur la Régénérations de Quelques Parties du Corps des Poissons. Historie de l'Acad. Roy. des Sciences.

Derwiler, S. R.

'18 Experiments on the Development of the Shoulder Girdle and the Anterior Limb of *Amblystoma punctatum*. Journal of Expt. Zoöl., 25.

Harrison, R. G.

'18 Experiments on the Development of the Forelimb of *Amblystoma*, a Self-differentiating Equipotential System. Ibid.

Mazza

1890 Sulla rigeneratione della pinna Caudale in Alcini Pesci. Atti. Soc. Ligust., Sc. 1.

Morgan, T. H.

'00 Regeneration in Teleosts. Arch. Entw.-Mech., 10.

'02 Further Experiments on the Regeneration of the Tail of Fishes. Arch. Entw.-Mech., 14.

'06 The Physiology of Regeneration. Jour. of Expt. Zoöl., 3.

Nussbaum and Sidoriak

'00 Beiträge zur Kenntniss der Regenerations—Vorgänge noch künstlichen Verletzungen bei älteren Bachforellen—Embryonen (*Salmo fario*). Arch. Entw-Mech., 10.

Ryder, J. A.

1882 Embryography of Osseous Fishes. Report of the Commissioner of Fisheries.

Weismann.

1892 The Germplasm: Translation by W. Newton Parker. New York.

[1] Only an abstract of the paper was read.

MICRODISSECTION STUDIES ON HUMAN SPERMATOZOA.

G. L. MOENCH, M.D., F.A.C.S., AND HELEN HOLT, B.S.

Although microdissection studies have been carried out by Barber (1, 2, 3), Tschachotin (4), Weber (5), Peterfi (6, 7), and others, and Chambers (8, 9, 10, 11), has given a comprehensive review of the subject, no such studies, it would seem, have been attempted on human spermatozoa. Through the courtesy of Dr. Robert Chambers, whom we want to thank here most sincerely, we were allowed the use of his laboratory and one of his microdissection apparatuses. We equipped this for our purpose with a special Leitz No. 28,340 substage condenser and a 10X and 25X compensation ocular, giving up to 2,640 diameters magnification.

Our original idea in making these studies was to determine whether double appearing cells were really double, or only two separate but contiguous or adherent cells, and whether other cell characteristics or abnormalities, such as apparent head caps, coiled tails, tapering heads, and so forth (see Fig. 1) could be produced artificially or dissected so as to give some clue as to their mode of origin.

Our results, due in part to the nature of the work itself, have thus far not been conclusive, but some rather interesting facts have nevertheless been met with.

We turned our attention first to the double forms, since it has been claimed that most of these are only apparent, and not real. In no case, however, did the dissection of such a double sperm cell prove it to be made up of two single and separate cells. The spermatozoa always proved to be attached to one another at one or more points, so that separation was impossible without injury to at least one of the cells. Even such cells as are shown in Fig. 1, 10, and Fig. 2, a_1 to a_4, have been proved to be of significance, since they show definitely incomplete separation. This strengthens the previously expressed view of the senior

author that this type of cell indicates some disturbance of spermatogenesis. We have also been able to dissect cytoplasmic masses off the base of the cell (Fig. 2, e_1 and e_2, and i) which suggests that such thickened membranes as are shown by Fig. 1, 7, are remnants of the cytoplasm, present at an earlier stage.

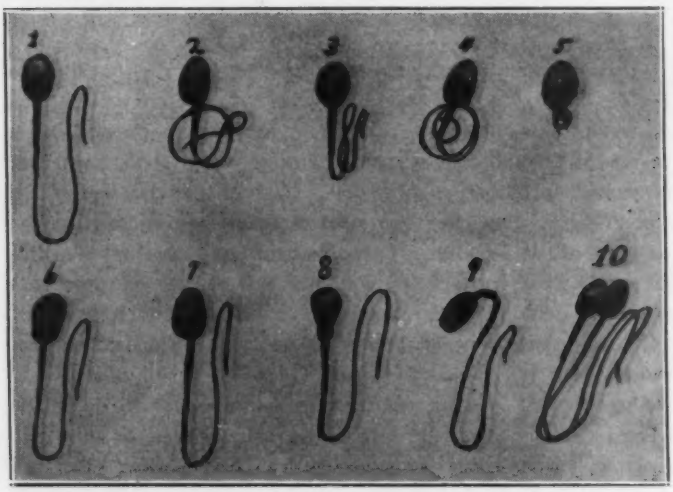


FIG. 1.

1. Normal human spermatozoön.
2. Spermatozoön with a coiled tail, undoubtedly due to purely accidental causes.
3. Spermatozoön of same class as 2.
4. Spermatozoön with type of coiled tail which may be an artefact or may be an actual change in the cell. It is at any rate a late change and of relatively little significance with reference to spermatogenesis. Such cells have been seen motile, although their motility is of necessity limited (see text).
5. Spermatozoön with coiled rudimentary tail. Such cells are useless as they have nothing to propel them.
6. Spermatozoön with head cap. Whether this represents a true head cap such as is found in the guinea pig or a separation of the cell membrane is still under investigation.
7. Spermatozoön with basal portion of cell membrane thickened. Remnant of cytoplasm?
8. Tapering spermatozoön head.
9. Spermatozoön with bent body.
10. Lack of complete separation of two sperm cells with small narrow heads.

That a cell with a bent body or middle piece is not an artefact, but represents some inherent disturbance of this region could also be definitely demonstrated, since we were able to straighten out such cells with the microdissection needles, and saw them assume their original deformity immediately upon the release from the needles. It is important to note here that live spermatozoa did not become immotile, as Peterfi (7) has described in the case of *Leishmania Tropica,* when touched by the microdissection needles, nor did the sperm cells exhibit any recognizable change.

Cells as depicted in Fig. 1, 4, were also investigated. This form of coiling of the tail had occasioned us some trouble of interpretation in our studies in human fertility. The coiling did not seem to be purely artificial, since this type of cell was usually motile in fresh semen specimens, and occurred sometimes with great frequency, even in the semen of normally fertile men. Thus this malformation could not represent a fundamental disturbance of spermatogenesis, but rather only a late change. After many futile attempts to produce this form of coiling, by heating, centrifugation, slow drying, and various reagents, we at last succeeded by using distilled water. This is especially remarkable because distilled water otherwise produced no visible effect upon the spermatozoön, and did not lead to any apparent change in the sperm head, while at the same time it caused both spermial body and tail to coil up in a half minute or less into a spiral as shown in Fig. 3.

Those sperm cells which had tapering heads were also interesting to dissect. In confirmation of the fact that such cell heads are often seen free without attached body or tail we found that the body in this type of cell could be broken off from the head by the micromanipulations more easily than in the normally shaped cell. Likewise the bodies of sperm cells kept for several days after emission broke off more easily than those of spermatozoa only two or three hours old. Perhaps this mechanical factor also has to be considered in explaining why spermatozoa retain their motility longer than their fertilizing power, since the tails might break off when such cells are called upon to overcome obstacles, for instance, to penetrate the ovum.

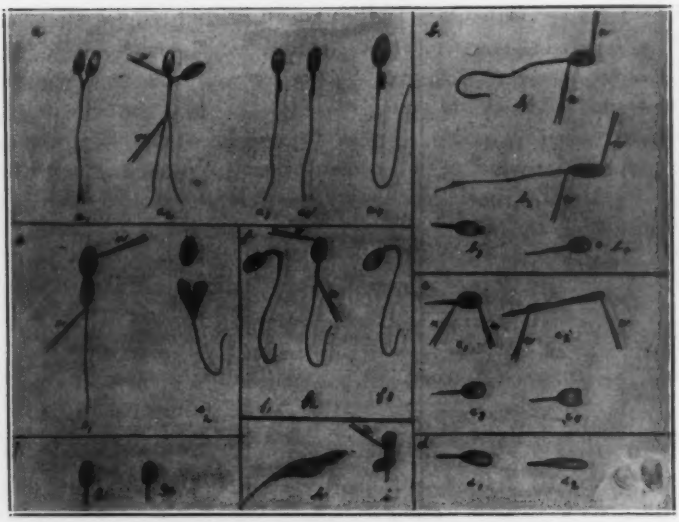


FIG. 2. In many of the spermatozoa only part of the attached body is shown. Unless specially shown, the body and tail were always normal in these cells.

a_1. Two spermatozoa closely attached to each other.

a_2. Microdissection needles (n and n) separating the two spermatozoa.

a_3. One spermatozoön completely separated from the other (a_2 and a_3^1) a_3 has a projection on its body, and a_3^1, a depression where the two cells were torn apart.

a_4. Represents a_3 at a higher magnification.

b_1. Normal motile spermatozoön grasped by the two microdissection needles and still motile.

b_2. Spermatozoön stretched with full tension on needles.

b_3. Small piece broken off at anterior end of the head which in breaking released the cell from the pull of the needles and it snapped back, assuming its original shape and the torn off fragment became spheroidal (b_4) (cell no longer motile after being stretched, see text).

c_1. Three day old non-motile spermatozoön, normal in appearance.

c_2. Extreme elasticity of head shown.

c_3. Normal shape regained immediately on releasing the needles.

c_4. Small piece of head broken off after several restretchings. Broken out piece not to be located.

d_1. Spermatozoön with tapering head (3 days old).

d_2. Cell after being stretched by needles did not regain former shape.

e_1. A spermatozoön with a swollen body (cytoplasm not cast off completely).

e_2. Mass dissected off with needles.

f_1. A motile spermatozoön with bent body.

An interesting and we believe hitherto unknown physical property of the sperm head is the elasticity which we discovered these cells to have. It is most marked in old, non-motile cells, but present to a certain degree also in perfectly fresh cells. Even the head of the live sperm cells seems, as far as we have been able to determine, rather elastic. Fig. 2, b_1 and b_2, for instance, represents a spermatozoön still actively motile held by the two needles. This sperm head stretched, as shown, until a piece broke off the anterior portion of the head, releasing it from the pull of the needles. The cell now was no longer motile, but we could not determine exactly when motility ceased. Due to the fact that the cell, to manipulate it, was pulled out of the main body of the small drop of semen to its shallow edge, the motility of the spermatozoön may also have been affected by the extreme shallowness of the seminal fluid at this point. We hope with more practice to be able to manipulate the cells in the deeper portions of the seminal drops. Old, non-motile cells stretched very far (Fig. 2, c_1 to c_4). While it is true that dead leucocytes can likewise be stretched to a considerable degree these white blood cells show no sign of the surprising degree of elasticity exhibited by the dead sperm heads. It must be remembered, however, that the sperm head consists probably entirely of nuclear material. Under all the conditions tried, even in perfectly fresh semen, the sperm head was found to be tough and viscid, and even when cut with the needles, gave no evidence of any escape of nuclear material. On the contrary, the cell injury tended to disappear, so that in a minute or two no evidence of the original injury was to be seen (Fig. 2, g_1 and g_2). Nevertheless, the repair of the lesion was often more apparent than real, because in pushing the head into various positions with the needle, the original gap might reappear, even if this area were not again

f_2. Same cell straightened out with the microdissection needles.

f_3. Same cell after release from the needles.

g_1. Head of motile cell 2 hours old with a gaping tear produced by a needle.

g_2. Gap has disappeared, cell again looks normal.

h. Unusual type of spermatozoön with much unshed cytoplasm, seen motile in a semen specimen.

i. Tapering sperm head surrounded at base by cytoplasm which has been dissected off on one side.

touched. In several cases, however, the size of the gap was distinctly smaller than the originally produced separation of the tissues. The elasticity of the sperm head substance was also shown by the fact that irregularly shaped pieces broken off from the head with the needles tended to assume a spheroidal, or at

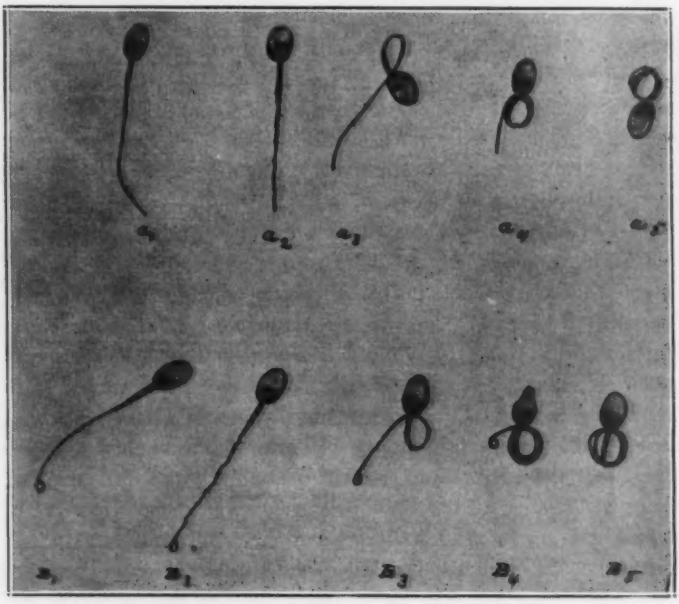


FIG. 3. Coiling of tails of sperm cells in distilled water.

a_1. Normal spermatozoön in seminal fluid.

a_2. Same cell a few seconds after it was pulled into distilled water by the microdissection needles. Tail fibrillating like frog's muscle in rigor mortis. Nevertheless this whole process of coiling of the tail must not be considered as a dying reaction of the cell as motile cells like a_1 to a_5 have been frequently seen (see text and Fig. 1).

a_3. First whip-like lash of tail.

a_4. Second convulsive jerk of tail.

a_5. Third stage of reaction. Tail completely coiled.

b_1 to b_5. Same stages in another normal spermatozoön which had, however, a small loop at the end of the tail from the start.

b_4. Cell head seen on edge.

It is easy enough to see how in cells like a_5 and b_5 the tail by folding over in the long axis of the head at the junction of the body and head may come to surround the spermial head.

least a more compact shape, and when stretched, showed an elasticity similar to that of the intact sperm head. It is worth noting, however, that a tapering cell after being stretched did not always contract to its former shape, and it would seem in general that this type of sperm head has less elasticity than normally shaped heads.

Our experiments thus far, at least prove that the various differences in size and shape of the sperm heads observed in seminal smears are really what they appear to be, and not due simply to external influences at work during the process of preparing and staining the sperm cells.

30 East 58th Street,
New York City.

BIBLIOGRAPHY.

1. **Barber.**
'04 Jour. Kansas Med. Soc., vol. 4.
2. **Barber.**
'11 Jour. Inf. Dis., vol. 8.
3. **Barber.**
'14 Phillip. Jour. Sci., Sect. B (Trop. Med.), vol. 9.
'15 Abstr. Ztschr. f. wiss. Mikros., vol. 32.
4. **Tschachotin.**
'12 Ztschr. f. wiss. Mikros., vol. 29.
5. **Weber.**
'23 Handb. biol. Arbeitsmethoden (Abderhalden), vol. II, p. 655.
6. **Peterfi.**
'23 Handb. microbiol. Techn., p. 2471, Berlin.
7. **Peterfi.**
'28 Arch. f. Protistenkunde, vol. 60, p. 492.
8. **Chambers.**
'18 Biol. Bull., vol. 34.
9. **Chambers.**
'22 Anat. Rec., vol. 24. Reprinted Jour. Roy. Micros. Soc., Dec., 1922.
10. **Chambers.**
'26–'27 General Cytology. Harvey Lectures, p. 41.
11. **Chambers.**
'21 The Microtomist's Vade Mecum. By Arthur Boles Lee. P. Blakiston's Son and Co., Philadelphia. Ed. 8.

SPERMATOZOÖN LIFE IN THE FEMALE REPRODUCTIVE TRACT OF THE GUINEA PIG AND RAT.[1]

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I. INTRODUCTION.

The problem of the vitality of the gametes is an important one, and especially in the mammals a problem that deserves additional study. The length of the life of the spermatozoa after introduction into the vagina at mating periods, the fate of excess sperm, and the influence of different physiological states of the uterus upon them are problems requiring additional consideration.

The work of Hammond and Asdell ('26) on germ cell viabil-

[1] This investigation has been aided by a grant from the committee on research in problems of sex of the National Research Council; grant administered by Prof. F. R. Lillie.

ity and fertility in the rabbit has done a great deal toward dispelling apparently erroneous conceptions promoted by statements from casual observations, often under conditions where the true state of affairs has been difficult to determine.

Stimulated by direct association with the study of Moore ('27, '28) on the viability of spermatozoa within the male tract, and at his suggestion, I have extended the study to the spermatozoa after their introduction into the female reproductive system. The rat and guinea pig have supplied the material for the experiment; and attention has been directed not only to the length of life of spermatozoa after introduction into the female at normal copulation, but also to spermatozoa after artificial introduction into the uterine tubes at inter-œstrum, both in the same species and in a foreign species. The results of this study are presented in the following pages.

I take this opportunity of expressing my indebtedness to Prof. Carl R. Moore for suggesting the problem, and for counsel and guidance during its progress.

II. Relative Amount and Motility, and the Location of Guinea-pig Spermatozoa in the Female Reproductive System after:

A. Normal Copulation.

Stockard and Papanicalaou ('17, '19) have clearly demonstrated the details of the œstrous cycle in the guinea pig. By noting the vaginal orifice one is able to select females as the period of œstrum approaches; and by introducing them into a cage containing the male animal, matings can be observed and the exact time of introduction of the sperm into the vagina recorded. Such females have been sacrificed at variable periods after copulation, and a routine search made for spermatozoa as follows:

Upon opening the body cavity dry slides were pressed against the ovary and surrounding region, normal saline was added to the smear, and an immediate microscopic search made for the presence of spermatozoa. Following this examination, the horns, oviducts, and ovaries were relieved from the posterior abdominal wall, and a hemostat placed at the junction of the oviduct and uterine horn. A hemostat was also placed at the bifurcation

of the horns and the body of the uterus. The ligature of these places prevented any sperm cells from passing out or contaminating one part or another. The oviducts were severed, placed in a depression slide containing normal saline, finely hashed with scissors, and the slide examined for the presence of spermatozoa. The uterine horns were examined differently. After cutting the horn from the body of the uterus, it was placed on a depression slide, cut longitudinally, normal saline added, and the contents scraped into the depression and examined. The horn was then transferred to another slide containing saline, but the epithelial side was inverted and allowed to stand until the previous slide had been examined. By this procedure it was hoped that most of the sperm were recovered.

The reproductive tract of thirteen female guinea pigs were examined as outlined above, between three and forty-five hours after witnessed matings. The observations on the spermatozoön condition in the various regions are presented in Table 1.

Since it was early appreciated that the motility of the spermatozoa was preserved for several hours after normal copulation, the majority of the observations were made during the second day. Reference to Table 1 emphasizes the fact that spermatozoa disappear from the vagina rather rapidly. One female examined at three hours after mating showed a few motile spermatozoa, but neither motile nor dead sperm were found in the vagina after nineteen hours in eleven cases examined.

Shortly after copulation quantities of spermatozoa that show vigorous motility can be recovered from the uterine horns. As the interval after mating is increased the vigor of motility can be seen to decrease. In nine females sacrificed on or before forty-one hours after mating motile sperm were recoverd from the uterine horns. In four animals sacrificed at forty-four and forty-five hours only a few non-motile spermatozoa were recovered.

The quantity of spermatozoa within the uterine horns diminishes fairly rapidly during the second day. I have been unable to satisfy myself as to the exact method of their elimination, but believe it to be largely due to the phagocytic action of cells within the lumen of the tract. One is able to note the collection of cells, probably leucocytes, about individual or several sperms. Continued

observation revealed that the head of the spermatozoa was the first to be taken in by the phagocytes. Indeed, it was very common to see the head of an engulfed sperm within the cell, while the tail, in some cases, remained free and moved back and forth. Such

TABLE I.

CONDITIONS OF THE SPERMATOZOA IN THE FEMALE GUINEA PIG AFTER NORMAL COPULATION.

No. of Animal.	Hrs. after Cop.	Relative Number and Motility of the Spermatozoa in:			Remarks.
		Vagina.	Uterine Horns.	Oviducts	
23	3	Few, feebly motile	Many present, highly motile	Few, feebly motile	
3A	14	Not examined	Many present, highly motile	Few, feebly motile	
17A	19	None present	Many present, highly motile	None present	
5	24	None present	Many present, highly motile	None found	
3	27	None present	Very few, feebly motile	Very few, feebly motile	Found 3 dead sperm in body cavity near ovary
15	30	None present	Few, highly motile	Very few dead	None found in body cavity
13	30	None present	Few, feebly motile	Few, feebly motile	Found dead sperm in body cavity near ovary
5A	35	None present	Few, feebly motile	Very few dead	Dead sperm in body cavity near ovary
6A	41	None present	Few, feebly motile	Very few, feebly motile	No sperm found in peritoneal cavity
15A	44	None present	Very few dead	Very few dead	No sperm found in peritoneal cavity
9A	45	None present	Very few dead	Very few dead	Few dead sperm found in body cavity
11A	45	None present	Very few dead	Very few dead	Some dead sperm found in body cavity
7A	45	None present	Very few dead	Very few dead	No sperm found in peritoneal cavity

observations appear to be in accord with those of Hoehne and Behne ('14) and other experimenters who believe that phagocytosis of the spermatozoa by leucocytes is largely responsible for

their destruction in many animals. Histological sections of the female tract were not made; therefore, I am unable to offer an opinion concerning the possible phagocytic action of the intact epithelium.

The fact that eleven females observed between nineteen and forty-five hours after mating failed to show the presence of living or dead spermatozoa in the vagina indicate that they are not removed by being passed from the tract externally.

Spermatozoa have been recovered from the oviducts three hours after mating. Motile sperm were found in the majority of the females examined forty-one hours or earlier, but later than this only non-motile ones were seen.

Examinations of the body cavity in five cases revealed dead spermatozoa. These had ascended through the uterine horn and oviduct, and passed through the slit-like communication between the ovarian bursa and the body cavity, Zuckerandl ('97). No motile spermatozoa, however, were recovered from the body cavity.

From these observations one may conclude that spermatozoa introduced into the female guinea-pig at coitus may retain their motility for forty-one hours. This is no implication that their fertilizing capacity is retained for this length of time.

B. Injection of Spermatozoa into the Guinea-pig Uterus at Œstrum.

Few, if any, attempts have been made to correlate spermatozoön activity with different physiological conditions of the uterus. It is an interesting question to enquire whether differences in viability might exist at different periods within the œstrous cycle, due perhaps to physiological conditions produced by the uterine rhythm.

Since the female guinea pig will not mate with the male except at œstrum, sperm suspensions were injected through the wall of the uterine horns after these had been exposed through a small aperture in the abdomen. Spermatozoa introduced into the uterus thus differed from their normal introduction in that no coitus occurred, an abdominal aperture and uterine wall puncture were experienced, and the spermatozoa were not mixed with the normal secretions of the seminal vesicles, prostate, nor Cow-

per's glands. Departing so radically from the ordinary course of nature it was thought necessary to compare the viability of the spermatozoa so introduced with the normal by making observations at œstrum. The effect of the unusual procedures should therefore be appreciated before comparisons are made with the

TABLE 2.

CONDITIONS OF THE SPERMATOZOA IN THE FEMALE GUINEA PIG AFTER INJECTION AT ŒSTRUM.

No. of Animal.	Hrs. after Inj.	Relative Number and Motility of the Spermatozoa in:			Remarks.
		Vagina.	Uterine Horns.	Oviducts.	
8A	19	Ligated	Few present, feebly motile	Few present feebly motile	
16A	20	"	Few dead	None found	
2A	24	"	Few, very feebly motile	Very few dead	Note animal No. 18, Table No. 2.
12A	26	"	Very few, feebly motile, few dead	Very few dead	Note animal No. 9, Table No. 2.
19A	30	"	Few dead	Few dead	
25	30	"	Very few dead	Very few dead	Sperm from same male inj. into No. 26 at same time
26	30	"	Very few dead	Very few dead	
20A	32	"	Many dead, 2 feebly motile	4 or 5 dead, few feebly motile	
23A	36	"	Very few dead, few feebly motile	None found	
28	40	"	Very few dead	3 or 4 dead sperm found	
21A	41½	"	Many dead, many fairly motile	Few dead	50% present in the horns were motile

inter-œstrous period. The procedure was as follows: Females in "heat" can be detected either with vasectomized males, or sometimes by the "touch" method and coitus be avoided. Such females were operated to expose the uterus and by means of a hypodermic syringe approximately ½ cc. of a sperm suspension was injected into each horn of the uterus. The vagina was ligated, usually to prevent discharge of the injected material but care was exercised to avoid hindrance to the blood supply. The sperm suspension was made by finely hashing two epididymides in a few

drops of physiological saline solution. Epididymal spermatozoa are thus obtained in high concentration, unmixed with seminal vesicle, prostate or Cowper's glands secretion. The female tract was examined for spermatozoa as after normal mating.

The results obtained from injecting such spermatozoön suspensions into the uterus of guinea pigs at œstrum are given in Table 2. Among eleven females sacrificed between nineteen and forty-one hours after injection, six showed motile sperm in the uterus, and the other five only non-motile cells. Motile spermatozoa were recovered from the oviducts in only two.

It is perhaps surprising to note that motile sperm were recovered from the uterine horns forty-one and one half hours after their artificial introduction by the above method. Since I was unable to find motile sperm for periods longer than this after normal copulation, it becomes apparent that epididymal sperm introduced at œstrum by injection through the uterine horns retain their motility for approximately the same length of time as those introduced in mating. It is interesting to note that spermatozoa taken directly from the epididymis, unmixed with the secretions of the seminal vesicles, prostate, and Cowper's glands, remained alive for as long a time as those mixed with the secretions of the accessory glands at normal coitus.

C. Injection of Spermatozoa at Inter-œstrum.

Since the persistence of motility of injected sperm was found to be of approximately the same duration as those introduced at coitus, one is able to study the effect of the uterine conditions at inter-œstrum. As the guinea-pig œstrous cycle has been shown to be approximately fifteen days in duration (Stockard and Papanicalaou), the mid or inter-œstrum was considered to be present about the eighth day after a definitely detected "heat" period.

Observations were made upon twenty-three female guinea pigs, into the uterus of which sperm suspensions had been injected, as described in section *B*. The results obtained are grouped in Table 3. Nine of the twenty-three females sacrificed between the fifth and fortieth hours after sperm injections showed motile spermatozoa in the uterine horns, and from six others motile sperm were recovered from the oviducts. Some non-motile sperm

TABLE 3.

CONDITIONS OF THE SPERMATOZOA IN THE FEMALE GUINEA PIG AFTER INJECTION AT INTER-ŒSTRUM.

No. of Animal.	Hrs. after Inj.	Relative Number and Motility of the Spermatozoa in:			Remarks.
		Vagina.	Uterine Horns.	Oviducts.	
4	5	Not ex. tied off	Many present highly motile	Not examined ligated	
10	10	Ligated	Few feebly motile, many dead	Not examined ligated	
12	10	"	Many motile, many dead	Not examined ligated	
10A	13	"	None found	Not examined ligated	
8	14	"	Few motile, many dead	Few highly motile	1 sperm vigorously motile in oviduct
14A	14	"	None motile many dead	Few dead, few feebly motile	
1	16	"	None found	Ligated	A very good injection was made
2	16	"	Few feebly motile, many dead	Very many, highly motile	
21	17	"	Very many dead	Ligated	
14	18	"	Very few present dead	Few present feebly motile	
19	19	None found	Few dead	Few dead	Sperm injected through the vagina
7	19	Tied off	Few feebly motile	Few feebly motile	
11	19	"	Few dead	None found	
6	20	"	Very few dead	Very few dead	
20	20	"	Very many dead	Very few dead	
22	20	"	Few motile, few dead	Few motile, few dead	
18	24	"	Very many dead	Very many dead	Sperm from same male also injected into animal 2A, Table 3
9	26	"	Many dead, few motile	Very many dead	Sperm from same male inj. into No. 12A, Table 3
22A	36	Ligated	Very many dead, very many motile	Two dead sperm found	
28	40	"	Few dead	None found	
31	40	"	Many dead	Few dead	
29	40	"	Many dead	6 or 8 dead, sperm found	
30	40	"	Very many dead	Very few dead	

were present in all. Thirty-six hours after injection, motile spermatozoa were recovered from the uterine horns; and in four females examined at the fortieth hour only dead cells were observed.

Animal number 22A, examined at thirty-six hours after injection, however, showed relatively large numbers of motile spermatozoa, and the motility was yet quite vigorous. It is quite certain that these sperm would have retained their motility for some time longer than thirty-six hours, but for how long is unknown. The difference of only five hours maximum duration of motility between sperm injected at œstrum and those injected at inter-œstrum does not appear to me to be of any real significance. So far as my results are concerned there is little or no indication of a characteristic differential survival time of spermatozoa introduced into the uterus at œstrum as compared with inter-œstrum.

D. Injection of Guinea-pig Spermatozoa into the Rat Uterus at Œstrum.

In order to obtain further knowledge on the behavior of guinea-pig spermatozoa they were injected into the uterine horns of the rat at œstrum. In this way the influence of the environment upon the life of the sperm could be studied.

The female rats were observed in the evening, for at that time the heat period is more likely to be detected. One female at a time was placed with a vasectomized rat, and the reactions observed. As soon as an animal came into "heat," it was operated and the uterus ligated at its junction with the vagina. In connection with this procedure it was noted that in every case the uterine horns were distended with a clear watery fluid. Long and Evans ('22) have described this condition at the œstrous period in the rat.

The sperm were taken from the epididymides of the guinea pig, mixed with a few drops of physiological saline to make about 1 cc., and half of this injected into each uterine horn.

A condensed outline of the results is given in Table 4. Nine female rats, into the uterine horns of which guinea-pig spermatozoön suspensions had been injected, were observed between the ninth and twenty-first hours after injection. Of the nine animals

TABLE 4.

INJECTION OF GUINEA-PIG SPERMATOZOA INTO THE RAT AT ŒSTRUM

No. of Animal.	Hrs. after Inj.	Relative Number and Motility of Spermatozoa in:				
		Vagina.	Left Horn.	Right Horn.	Left Oviduct.	Right Oviduct.
5A	9	Ligated	Many dead	Very many dead	Many dead	Many dead
6A	10	"	Very few dead	Few dead	Many dead, 1 feebly motile	Many dead
3A*	11	"	Very few dead	Very few dead	None found	None found
4A	11	"	Few dead	Many dead	2 or 3 dead	Many dead
7A	11	"	Few dead	Few dead	Many dead, 2 feebly motile	Few dead
8A	13	"	Many dead	Many dead	Few dead	None found
9A	14	"	Few dead	Many dead	Few dead	Few dead
1A†	20	"	Very few dead	Very few dead	None found	None found
2A†	21½	"	None found	None found	None found	None found

Note: the uterine fluid was withdrawn, before the sperm were injected, from the left horn of all animals except 1A, 2A, 3A.

* The fluid was withdrawn from both horns.

† The fluid was not withdrawn from either horn.

examined only two were found to possess motile spermatozoa, one at the tenth and at the eleventh hour. In each case, however, only one or two cells were found that showed any movement, and this was very slight.

Not only are the spermatozoa of the guinea pig rapidly killed after introduction into the rat uterus, but also the dead sperm are rapidly removed from the horns. Large masses of dead sperm were obtained from the guinea-pig uterus up to forty hours, but after injection of similar guinea-pig sperm suspensions into the rat uterus they are practically all removed within one half this time.

These findings clearly show that the environment (uterus) has a remarkable effect upon the spermatozoön life, for whereas a guinea-pig sperm suspension injected into the guinea-pig uterus shows some motility up to about forty hours, with quantities of dead sperm remaining, similar suspensions injected into the rat uterus is followed by loss of all motility within approximately eleven hours, and a rapid removal of dead spermatozoa.

III. Relative Amount and Motility, and the Location of Rat Spermatozoa in the Female Reproductive System after:

A. Normal Copulation.

Experiments with the rat which duplicated those on the guinea pig were conducted for the purpose of comparing the length of spermatozoön life in the two.

Normal copulations were witnessed, the time recorded, and the females sacrificed as desired for observation. My observations on the persistence of motility of spermatozoa in the rat after normal coitus are recorded in Table 5. Various parts of the reproductive system were clamped off, and search for spermatozoa was conducted as in the case of the guinea pig.

The female rat was not examined earlier than twelve hours after mating. Fourteen animals were sacrificed between twelve and twenty-three hours after copulation. Motile sperm were recovered from only four animals, and dead cells from all but one. The latter was not observed until twenty-three hours after mating.

TABLE 5.

CONDITION OF THE SPERMATOZOA IN THE FEMALE RAT AFTER NORMAL COPULATION.

No. of Animal.	Hrs. after Cop.	Relative Number and Motility of Spermatozoa in:			Remarks.
		Vagina.	Uterine Horns.	Oviducts.	
1	12	Not examined	Very many, highly motile	Very many, highly motile	
2	13	None found	Few dead	None found	
3	13	Very few dead	None found	None found	Vaginal plug found
4	14½	Very many motile	Few dead	Few dead	Vaginal plug present
5	16	Few dead	None found	None found	The horns were distended with fluid
6	16	Many dead	Many dead	Very few dead	Few dead sperm found in body cavity near ovary.
9	16	Few dead	None found	None found	Both horns filled with fluid when examined
11	16	Many dead	One dead sperm in right horn	Many dead, few highly motile	
10	16½	None	Many dead	Few dead	
14	17	Few dead	Few dead	Few fairly motile	
7	20	Many dead	Few dead	Few dead	
12	20	None	Few dead	Few dead	
8	21	Many dead	Few dead	Few dead	
13	23	None	None found	None found	Both horns were filled with fluid when examined

Motile sperm were recovered from the oviducts sixteen and seventeen hours after copulation, from the vagina at fourteen and one half hours in one animal, and from the uterine horns and oviducts in larger quantities and more vigorously motile at twelve hours after mating.

B. Injection of Rat Spermatozoa into the Rat Uterus at Œstrum.

Rat spermatozoa were injected into the female rat at œstrum in order to compare the conditions found after normal copulation with those caused by the injection of the sperm, and also to compare the results of injecting the sperm into the guinea pig.

One female rat at a time was placed with a vasectomized male, and as soon as an animal was found to be in "heat" the rat sperm were injected. The injections consisted of a spermatozoön suspension made by finely hashing two epididymides in a few drops of normal saline. The resulting concentrated sperm suspension was then strained through a piece of sterile gauze, and drawn into a hypodermic syringe. The volume of sperm and saline thus obtained was about 1 cc. The female was opened and the horns were ligated near their junction with the vagina, care being taken to avoid interference with the blood supply of the genital tract. About ½ cc. of this sperm suspension was injected into each horn, and later the animal was killed for examination.

Table 6 shows a record of the observations made, and it will be noticed that motile spermatozoa were found up to twelve and one half hours after injection as compared to seventeen hours after normal copulation. Evidently rat spermatozoa are more sensitive to injection than guinea-pig sperm, for there is a difference of four and one half hours in the length of time they were found alive. It was found that injection of guinea-pig spermatozoa into the guinea pig did not show this percentage difference.

The uterine fluid was removed from only one animal, 2*B*, and that from the left horn. However, in this case dead spermatozoa were foud in the right oviduct, but none were found in the left one. This indicates that the uterine fluid may assist the sperm in their ascention toward the ovary. It does not necessarily preserve their life, for motile sperm were found in the left horn while only dead ones were found in the right horn.

TABLE 6.

INJECTION OF RAT SPERMATOZOA INTO THE RAT AT ŒSTRUM

No. of Animal.	Hr. after Inj.	Relative Number and Motility of the Spermatozoa in:				
		Vagina.	Left Horn.	Right Horn.	Left Oviduct.	Right Oviduct.
1B*	11½	Ligated	Many dead	Many dead	Few dead, 1 motile	Few dead
2B†	12½	"	Many dead, very few motile	Few dead	None	Few dead
3B*	12½	"	Few dead	Few dead	Few dead	Few dead
4B*	15	"	Few dead	Few dead	Few dead	3 or 4 dead
5B*	15	"	Few dead	Many dead	Few dead	None found
6B*	15	"	Many dead	Many dead	Many dead	Few dead

* No fluid was withdrawn from the uterine horns before the spermatozoa were injected.

† The fluid was removed from the left horn, but not from the right horn before the spermatozoa were injected.

C. Injection of Rat Spermatozoa into the Guinea-pig Uterus.

Suspensions of rat spermatozoa, prepared as above outlined, were injected into the guinea-pig uterus to study the effect of a foreign uterine environment upon their motility.

TABLE 7.

INJECTION OF RAT SPERMATOZOA INTO THE GUINEA PIG AT ŒSTRUM AND AT INTER-ŒSTRUM.

No. of Anim.	Hrs. after Inj.	Relative Number and Motility of Spermatozoa in:			Time of Injection in Sexual Cycle.
		Vagina.	Uterine Horns.	Oviducts.	
25A	4	Ligated	Many dead, 2 or 3 motile	Few dead	At œstrum
32	4½	"	Many dead, 2 or 3 motile	Few dead	At inter-œstrum
1B	6	"	Many dead	5 or 6 dead	" "
2B	6	"	Few dead	Many dead	" "
3B	6	"	Many dead	Few dead	" "
24	6	"	Many dead	Many dead	At œstrum
33	6	"	Few dead	Many dead	" "
34	6	"	Many dead	Many dead	" "
26A	6	"	Many dead	Few dead	" "

Nine female guinea pigs (five at œstrum and four at inter-œstrum) were injected with rat spermatozoön suspensions, and the reproductive tract examined between four and six hours after the injection. The record of observations given in Table 7 consists of the nine injections at the two different stages of uterine development.

Two animals of the nine examined revealed motile sperm at four and four and one half hours. Seven females examined six hours after injection showed dead spermatozoa in both the uterine horns and the oviducts, but none were found alive.

Since rat spermatozoa injected into the rat uterus in the same manner as those injected into the guinea pig were found to possess motility for 12½ hours whereas in the guinea-pig uterus motility survived for less than six hours, it is evident that the species foreign uterus does present an environment in which motility is more rapidly lost.

IV. Discussion.

Several, possibly the majority, of the current textbooks of gynecology, embryology, and obstetrics state that human spermatozoa retain their motility in the female reproductive tract for three and a half weeks. So far as I can ascertain such statements are based upon the report of Duhrssen ('93) who claims to have found twelve motile sperm in a diseased Fallopian tube removed nine days after admission of the patient to the clinic; and according to the patient the last coitus had occurred three and one half weeks previous. Strict dependence cannot always be placed upon such verbal statements; and it would appear that in the absence of substantiation of such reports from carefully conducted investigation, the idea if untenable should be relegated to history.

After a brief survey of the length of life of spermatozoa in some of the lower vertebrates, some data pertaining to spermatozoön life in the human female will be given. Readily admitting the risk of drawing conclusions for one animal species on data obtained from a different species, one should not be deterred from conducting sufficient investigation to appreciate the general biological principles underlying the question.

My own observations upon laboratory mammals have convinced me that spermatozoa introduced into the female guinea-pig reproductive tract at a normal mating do not retain their motility for longer than approximately 40 hours. I have found them consistently up to 40 hours, but in ever diminishing numbers and intensity of motility as this period is approached. Forty-one hours is the maximum time motility was observed in the guinea pig, and beyond this time only non-motile sperm were observed.

In the rat, spermatozoa live for even a shorter time after copulation. In one case examined 17 hours after mating a few motile spermatozoa were present, but in the majority of cases examined 12 to 16 hours post-coitus, the cells were present but non-motile.

Turning to the literature dealing with similar studies on mammals, other than man, we find many similar experiences. Hensen ('81), cited by Hoehe and Behne, stated that sperm in the guinea pig were immotile 16 hours after copulation. It is certain, how-

ever, that a more extensive study would have extended the period of life, as my experiments show. In the mouse Sobotta ('95) claims that spermatozoa live only 9 to 10 hours and are then absorbed by the uterus. In the rabbit Hammond and Asdell ('26) showed that the spermatozoa were motile and capable of fertilizing the ovum for but 30 hours. The maximum persistence of motility was not determined. They state: "With regard to the time differences observed between the loss of motility of the sperms and the loss of their power to fertilize ova, the writers are not inclined to think that there is any abstruse mechanism involved in these differences whereby the fertilizing power is destroyed without affecting the motility. The results given above suggest rather that difficult conditions which exist for the sperm in the female passages, which allow of a life of 30 hours instead of 33 days (in the male tract), are sufficient to kill off sperms of low vitality under 10 hours, the time at which ovulation normally occurs after coitus in the rabbit."

Hoehne and Behne ('14) found a few motile sperm in the uterus of a rabbit two days post-coitus, but none were found in the oviducts. In the mare, Anderson ('22) found vigorously motile sperm in the uterus and tubes 7¼ hours post-coitus. It is, however, not known how long the spermatozoa would have lived. His experiments and observations on the persistence of life of spermatozoa lead him to believe that they rarely live longer than 48 hours in the female tract.

Lewis ('11) sacrificed 25 sows after normal matings, and examined them for the distribution and persistence of sperm. In three cases only could live spermatozoa be found for periods longer than 20 hours after coitus. One showed a few motile cells at 41½ hours.

In all literature reports, with which I am familiar, the persistence of spermatozoa in the female genital tract of lower mammals all agree in one general respect, namely: that the life of sperm after introduction into the female tract is to be reckoned in terms of a few hours (30 to 40 hours). One outstanding exception for mammals (the bat), and conditions found in fowl are to be noted. Several different accounts of copulation and fertilization in the bat agree in the general idea that copulation occurs

before the winter hibernation, that the spermatozoa remain alive, though quiescent, over winter and fertilize the ovum during the following spring. The account of Courrier ('27) is an extensive review of this general situation. In the bat conditions are complicated by the hibernating state: the spermatozoa apparently exist in quiescent clumps, and are not in a state of motility; hence, the persistence of life in such a state, and in an organism at a low metabolic level, is on a different plane from the rapid and continuously motile sperm in the uterus of the average mammal. In other words, the sperm are in a state similar to that of their quiescence in the male genital tract.

In the fowl, it has been generally stated that fertile eggs may be laid for a period of approximately two weeks after isolation of the cock. However, in the duck Chappellier ('14) found absolute sterility from the 7th to 11th day. In the case of fowl, as will be discussed later, the testis is abdominal and the natural temperature at which the sperms are formed is similar to that of the oviduct, which is not the case in most mammals where the scrotal temperature is lower than the abdominal temperature.

If we examine the data on spermatozoön motility in the human female tract we have the older conception of Duhrssen ('93) that they remain alive for three and one half weeks. Pertaining to the report of Duhrssen, Hoehne ('14) states: "such vital energy in the sperm is rather unusual in my opinion. It may be explained by the lack of reaction (phagocytosis of the sperm by leucocytes) in the diseased Fallopian tube." Bossi ('91) refers to a case in which the sperm are said to have survived over 12 days. Nürnberger ('20) demonstrated motile spermatozoa in normal Fallopian tubes, which had been removed 13 or 14 days after the last stated cohabitation. Strassmann ('95) records the occurrence of living sperm in the human female a week after the last coitus. Triepel ('14, '19) assumes that the spermatozoa live only a short time in the female tract.

Huhner ('28) states that sperm lose much of their motility within 15 minutes and are dead within 4 hours in the vagina; and that many cases of sterility are attributed to the fact, under certain conditions, that the spermatozoa are rendered immotile by the unfavorable conditions of the vagina before they reach the

cervical canal. Hoehne and Behne ('14) made a thorough study of sperm motility in the human and found that in most cases the spermatozoa were killed within one hour in the vagina and within 2 or 3 days in the uterus and tubes. They conclude that there is no evidence that the sperm live longer than 3 days in the normal human female uterus.

Runge ('09) also conducted an extensive experiment on this problem and agrees very closely with the latter's results. He conducted 32 observations on 17 women (non-gravid) and found that the sperm are killed much quicker in the vagina than in other parts. He was able to find live spermatozoa in the uterus 36 hours post-coitus in a few cases, but failed to observe living spermatozoa in the uterus after 3 days.

The fact that usually the assertion of the individual as to the time of the last coitus is the basis upon which the length of life of the spermatozoa in the tract, where recovery and motility have been noted so long, give the feeling that many reports have to be discounted. Such reliance may account for so many conflicting statements.

A biological fact that has but recently been appreciated is the deleterious effects body temperature has upon the sperm. Moore ('28) and Benoit ('26) have shown that guinea-pig spermatozoa in the tail of the epididymis will remain alive and capable of motility for as long as 65 days, provided the epididymis remains in the scrotal sac and subject to its normal heat regulating properties. Moore ('26) and Heller ('29), working under Moore's direction, have shown, however, that after the same operation if the epididymis is elevated into the abdomen where it is subjected to the normal body temperature the sperm remain alive for but 14 days.

In the rabbit Hammond and Asdell ('26) found that the average scrotal temperature taken from a number of bucks was 3° F. lower than the abdominal temperature. The general body temperature, therefore, reduces the life of sperm, even when they are confined within the male reproductive tract and in a quiescent state amid the secretion material normal for them.

Comparing the life in the male tract with my findings in the female tract in which the spermatozoa retain their motility for but 40 hours, even when introduced with the male secretions at copu-

lation, one appreciates that their life is greatly curtailed. This is not due alone to the action of body temperature, which must be recognized as a factor, but to constant and vigorous motility tending to exhaustion, and to the action of phagocytes, and possible uterine secretions harmful to them.

Considering all these elements and the report of more recent observations in the human, it is probable that we will have to consider motility in the human female tract as more truly being expressed in terms of a few days (2 to 3 days) than in terms of weeks as most textbook accounts indicate. Again, it should be emphasized that we are speaking of mere capacity to show motility and not fertility. Lillie ('15) emphasized that the fertilizing capacity of invertebrate sperm is lost considerably earlier than motility. However, Hammond and Asdell ('26) believe that in the rabbit loss of fertility and motility are closely related.

On general principles one might assume that the changes in the uterus would be such as to favor the retention of motility of the spermatozoa at the time they would normally be introduced at mating, or conversely, that the uterus at inter-œstrum would be less favorable for the male sex cells. My observations, however, do not support such a conception. Spermatozoa introduced into the uterus at œstrum either by normal copulation or by introducing them with a hypodermic needle through the uterine walls have been seen to retain their motility for 40 hours. When they were introduced at inter-œstrum they were observed to retain their motility to 36 hours. The difference of four hours in duration of motility in the uterus at œstrum and inter-œstrum is so small that I do not consider it of importance. At 36 hours movement of a fairly vigorous type in a fair percentage of the remaining spermatozoa leads me to believe that more cases examined would serve to extend somewhat the maximum period of motility observed.

To emphasize the variable period of sperm motility in different species it may be recalled here that guinea-pig spermatozoa remain motile after coitus for longer periods than do rat spermatozoa. The maximum periods observed by me were 41 hours in the guinea pig and 17 hours in the rat. One must exercise caution in making too strict an application of the period of motility retention in one animal to the possible length in an animal of another species.

Hoehne ('14) and Hoehne and Behne ('14) injected foreign and species specific sperm to study the persistence of motility. They do not give the procedures or methods but a brief summary of their results may be noted. Hoehne ('14) states that if rabbit sperm are injected into the guinea pig and vice versa, after 9 hours there are many dead sperm in the uterus and tubes, after 2 days the number of motile sperm is less; after 4 days there are only a very few living spermatozoa. However, if the sperm are injected into the body cavity they are eliminated within 4–20 hours by phagocytosis. Hoehne and Behne ('14) injected human spermatozoa into the rabbit uterus, and after 3 or 4 days the findings were negative; in one case one live sperm was found 18 hours after the injection. When rabbit sperm were injected into the rabbit most of the sperm were dead after 2 days, and all were dead after 6 days. After normal coitus in the rabbit a few motile spermatozoa were found in the uterus after 2 days.

Perhaps the guinea pig and rabbit are more closely related in this respect than the guinea pig and the rat, but it is interesting to note the effect of a strange uterine environment on the persistence of sperm motility. In my experiments guinea-pig spermatozoa injected into the guinea-pig uterus with a hypodermic needle have been found to be motile for 40 hours. Similar guinea-pig sperm injections into the rat uterus, however, showed motility for but 11 hours as a maximum. A simliar reduction in the length of time spermatozoa remain motile was seen when rat sperm suspensions were injected into the rat uterus and guinea-pig uterus. Rat sperm injected into the rat uterus were observed to be motile for approximately 12 hours, whereas in the guinea-pig uterus the maximum period of motility observed was 4 hours. The effect of a foreign species uterus on spermatozoön motility is possibly due to enhanced phagocytic action and possible to incompatible secretions produced by the uterus.

The fact that there is such a pronounced effect of the uterine environment on spermatozoa from a different species seems to indicate that radically different physiological uterine states should express themselves by affecting differentially the length of persistence of motility. The fact that guinea-pig spermatozoa injected into the guinea-pig uterus at œstrum and at inter-œstrum

retain their motility for similar periods of time affords additional evidence that the inter-œstrous uterus is not sufficiently different physiologically from the uterus at œstrum to have a detectable effect upon spermatozoön life.

V. Summary and Conclusions.

1. A few feebly motile spermatozoa were found in the uterine horns and oviducts of the guinea pig up to 41 hours after normal copulation.

2. Motile sperm were observed in the horns of the guinea pig uterus 36 hours after injecting guinea-pig sperm into the uterus with a hypodermic needle at inter-œstrum.

3. Live sperm were found 41½ hours after injecting guinea-pig spermatozoa into the guinea-pig uterus at œstrum.

The fact that live sperm were observed 5½ hours longer after their injection at œstrum than inter-œstrum does not signify that the physiological conditions at œstrum are more favorable than at inter-œstrum.

4. Guinea-pig spermatozoa were observed to be motile for only 11 hours when injected into the rat uterus.

5. Motile sperm were found in the oviducts 17 hours post-coitus in the rat.

6. Rat spermatozoa injected into the rat uterus with a hypodermic needle were observed to retain motility for 12½ hours.

7. Rat sperm remained motile for but 4½ hours when they were injected into the uterus of the guinea pig.

8. It appears that a non-species uterus has a marked effect upon the destruction of spermatozoa.

9. No physiological difference between the uterus at œstrum and inter-œstrum was detected by using the duration of spermatozoön life as the indicator.

VI. BIBLIOGRAPHY.

Anderson, W. S.

'22 Vitality of Spermatozoa. Kentucky Agricultural Expr. Station Bull., No. 239.

Benoit, J.

'26 Recherches anatomique cytologique et histophysiologiques sur les voies excretrices du testicule chez les mammiferes. Arch. d'anat., d'hist. et d'embryologie, vol. 5: 173–412.

Bossi.

'91 Quoted by Marshall and Jolly: Contributions to the Physiology of Mammalian Reproduction. Philosophical Trans., 1906 (198B).

Chappellier.

'14 Quoted from Hammond and Asdell. (See reference.)

Courrier, R.

'27 Etude sur le determinisme des characteres sexuels secondaires chez quelques mammiferes activite testiculaire periodique. Arch. de Biol., 37: 175–334.

Duhrssen.

'83 Quoted from Hoehe ('14) and Hoehne and Behne ('14).

Hammond, John, and Asdell, S. A.

'26 The Vitality of Spermatozoa in the Male and Female Reproductive Tracts. British Jour. Exp. Biol., 4, No. 2, 155–185.

Hammond and Marshall.

Reproduction in the Rabbit. Biological Monographs and Manuals (Oliver and Boyd: London).

Heller, R. E.

'29 New Evidence for the Function of the Scrotum. Physiological Zoölogy, vol. II, No. 1, Jan., 1929.

Hoehne, O.

'14 Experimentelle Untersuchunger über das Schicksal arteigener und artfremder Spermatozoen im weiblicken Genitalapparat und in der Bauchhöhle. Deutsche Gesellschaft Für Gynec., 1914, pp. 514–516.

Hoehne, O., and Behne, K.

'14 Über die Lebensdauer homologer und heterologer Spermatozoen im weiblichen Genitalapparat und in der Bauchhöhle. Zentble. für Gynec., 38, No. 1, pp. 5–20.

Hensen.

'81 Cited by Hoehne and Behne.

Huhner.

Quoted by Meaker. Journal American Medical Assn., vol. 90, No. 2, January 14, 1928.

Lewis, Lowery L.

'11 The Vitality of Reproductive Cells. Oklahoma Agricultural Expr. Station Bull. No. 96.

Lillie, F. R.

'15 Studies of Fertilization, VII. Analysis of Variations in the Fertilizing Power of Sperm Suspensions of *Arbacia*. Biol. Bull., 28.

Moore, Carl R.

'26 The Biology of the Mammalian Testis and Scrotum. Quart. Review in Biol., vol. I, pp. 4–50.

'28 X. Spermatozoön Activity and the Testis Hormone. Jour. Expr. Zoöl., 50, pp. 455–494.

Nürnberger.

'20 Quoted from Williams' Obstetrics, fifth edition, revised.

Runge, E.

'09 Beitrag zur Aetiologie und Therapie der weiblichen Sterilität. Archiv. für Gynec., 87: 1909, pp. 572–585.

Sobotta, J.

'95 Arch. Mikr. Anat., 46, 15. Also quoted by Hoehne and Behne.

Strassmann

'95 Quoted from Marshall and Jolly.

Stockard and Papanicalaou.

'17 The Existence of a Typical Œstrous Period in the Guinea Pig with a Consideration of the Histological and Physiological Changes. Amer. Jour. Anat., 22, No. 2, Sept., 1917.

Stockard and Papanicalaou.

'19 The Vaginal Closure Membrane, Copulation and the Vaginal Plug in the Guinea Pig, with Further Consideration of the Œstrous Rhythm. Biol. Bull., 37, No. 3.

Triepel.

'14 Anat. Anz. Xlvi; (1915) Xl viii; (1919) lii. Quoted from Hammond and Marshall.

Zukerandl, E.

'97 Zur vergleichenden Anatomie der Ovarialtaschen, Anat., Hefte, Wiesb., Bd. 8, S. 705–799.

THE CORRELATION OF THE AMOUNT OF SUNLIGHT WITH THE DIVISION RATES OF CILIATES.

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The analysis of the division rates of ciliates of three diverse orders which had been cultured under practically identical conditions, disclosed only a secular trend and a yearly cycle of change. The maximum of the yearly cycle [1] occurred during the month of July and the cycles of each organism were similar. The slopes of the secular trends were different for each organism. No evidence of the special "cycles" and "rhythms" which protozoölogists had reported in other investigations was found. This paper will show that the cycle of seasonal variation is closely related to the amount of sunshine recorded during the cycle and may perhaps be determined by solar radiation. Evidence from other investigations will be cited to support this conclusion.

I.

The division rates of *Paramecia aurelia* (mutant), *Blepharisma undulans,* and *Histrio complanatus* grown in pedigree isolation culture, with the same culture media, for three years, were obtained by Dawson.[2] These data were used in a previous analysis by Richards and Dawson.[3] The monthly averages of the sunlight recorded at the Boston and Block Island Stations of the U. S. Weather Bureau in terms of the per cent. of possible sunlight were furnished to me through the courtesy of Mr. G. A. Loveland. The figures for the Block Island Station for July and August were used as being the best available representation of the sunlight at Woods Hole, where Dawson kept the protozoa during

[1] *Rhythm* and *cycle* are here used with their usual meaning. The terms will be enclosed in quotation marks when given the special and restricted meaning found in the protozoölogical literature.

[2] Dawson, J. A., 1926, *J. Exp. Zoöl.*, XLIV., 133; 1926–27; XLVI., 345.

[3] Richards, O. W., and Dawson, J. A., 1927, *J. Gen. Physiol.*, X., 853.

the summer. The per cent. of possible sunlight, for each month, is the ratio of the recorded number of hours of sunlight to the maximum number of hours of sunlight that could occur that month if there was no cloudiness, etc. The per cent. of possible sunlight is used here because it saves the first step of the analysis, namely the converting of the number of hours into percentages.[4] This method includes and emphasizes the cycle of sunlight variation and introduces no significant error.[5]

The average yearly cycle of seasonal variation for each organism is shown in Fig. 1. The statistical methods used in obtain-

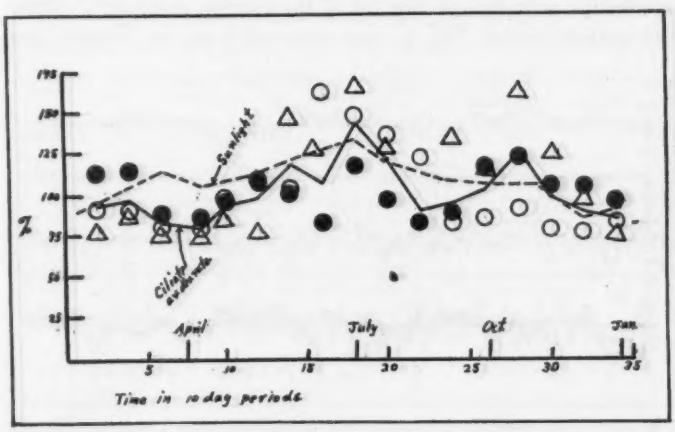

FIG. 1. The yearly cycle of seasonal variation for each organism and the average amount of sunlight. Note: maxima in July. o, *Paramecium*. ●, *Blepharisma*. △, *Histrio*.

ing this cycle, and employed for the further analysis of the sunlight data, are the same and are described in the previous paper.[3] The variation in the amount of sunlight is very similar to the variation in the magnitude of the average division rate of the

[4] *Cf.* previous analysis[3] and Rietz, H. L., Handbook of Mathematical Statistics, New York, 1924, 151 ff.

[5] When the means of the curve of percentage of possible sunlight and the curve of total hours of sunlight are superimposed the deviations of the monthly values from each other average 2.6 per cent. which is only 0.4 unit of Fig. 2*c* and does not effect the calculations. The mean absolute deviation is — 0.36 per cent. which shows that since the deviations almost cancel each other they may be ignored in this analysis.

organisms. Since the amount of sunlight for any given month was different in each year, as is shown in Fig. 2*a*, an accurate comparison can only be made by first removing the amount of the division rate cycle that corresponds to the amount of sunlight for each time and then comparing the residues. This is the procedure that was used in the original analysis of the data, except that this time we use the recorded amount of sunshine for each month instead of a generalized statistically determined cycle to eliminate the observed cyclic variation in the division rates which have been corrected for the secular trend; Fig. 2*b*. (This figure is identical with that of Fig. 2*d* of the original analysis.[3]) After the sunlight cycles, Fig. 2*a*, are removed from the division rate

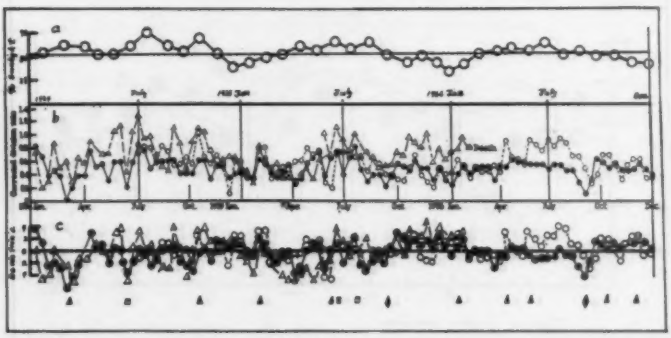

FIG. 2. *a*, the amount of sunlight. *b*, the division rate corrected for secular trend (*Cf.* Fig. 2*d*, Richards and Dawson[3]). *c*, division rate residues after minimiznig secular trend and variation associated with sunlight. o, *Paramecium*. ●, *Blepharisma*. Δ, *Histrio*. ▲, deviations associated with a change of water source. ■, deviations associated with removal to Woods Hole or Cambridge. ◆, deviations associated with temporary change of technician during the absence of Dawson.

data, Fig. 2*b*, there is left a residual amount of variation which is plotted as deviations from the superimposed mean division rate for each organism, in Fig. 2*c*.

II.

The residual curves may now be directly compared, as the disturbing effect of trend has been eliminated. If the amount of solar radiation determines the yearly cycle of seasonal variation

in the division rates, all relation of the division rate curve of each organism to those of the others will have disappeared and the remaining deviations of the rates will be due to other influences not completely controlled by the culture technique.

The maximum correlation of the corrected *Paramecium* rates (x) with the *Blepharisma* rates (y) is their partial correlation independent of time, and is $r_{xy.t} = -.001$. The *Histrio* rates (z) may be correlated with the composite of the *Paramecium* and

TABLE I.

THE LEXIAN RATIOS* OF THE DIVISION RATES.

Organism.	Original Data.	Original Corrected Data.	Final Original Data.†	Corrected for Sunlight.	Final Corrected for Sunlight.†
Paramecium....	2.19	1.54	1.19	1.33	1.15
Blepharisma....	1.81	1.61	1.31	1.28	1.07
Histrio.....	3.28	1.50	1.21	1.51	1.44

* The ratio of the relative standard deviation of the series to the relative Bernoulli standard deviation for the same series.

† These columns are for the corrected data less those deviations due to known disturbances (*Cf.* legend Fig. 2).

Blepharisma rates by means of the multiple correlation $r_{z.xy} = 0.08$. These coefficients demonstrate no relation between the corrected division rates. The unifying effect of the seasonal variation is removed. This removal is more complete than the removal of the statistically determined cycle of the previous analysis because the correlation coefficients of the original analysis [3] were -0.08 and 0.29, respectively. Consequently, the amount of sunlight seems to have ordered the similar yearly cycle of variation in the division rates of these representatives of three different orders of the ciliate infusoria.

III.

By means of the Lexian ratio the deviations of the residual curves of the previous analysis [3] were examined to disclose whether or not these remaining variations were due to chance experimentally uncontrolled factors alone or if they were due to definite unifying effect. The numerical values of the Lexian ratio

are given in Table 1. When the deviations known to be caused by a change of medium, by removal from Cambridge to Woods Hole, or return, or by changes in culture technique are omitted the Lexian ratios more nearly approached unity. Purely chance deviations of a constant probability would give a Lexian ratio close to unity.

The Lexian ratios for the data of Fig. 2*c*, after the variations associated with the amount of sunlight are removed, are less than the figures of the previous analysis and, after the disturbances correlated with known causes are removed, the ratios are very nearly unity for all but the *Histrio* data. This probably indicates the inability of the *Histrio* to become adapted to the environment of the cultures, which ultimately resulted in its death.

The residual variation of the *Paramecium* and *Blepharisma* rates shows little disturbance beyond what might be attributed to chance influences. It is possible that if a corresponding ten day average, or, better, a running average of the sunlight for ten-day periods, were available and were used in place of the monthly averages of the sunlight, more of this remaining variation might have been removed. This analysis of the data leaves no trace of "cycles" or "rhythms" that might be attributed to cellular reorganization; and in fact none were observed by Dawson.[6] Consequently, it is an external influence, connected with sunlight, and not an internal organization that makes the inherently different division rates follow a uniform seasonal course. The division rate of the *Blepharisma* follows the amount of sunlight more closely than the others which may be due to the greater absorption of sunlight by the pigment of this organism.

IV.

This analysis shows that the yearly cycle of seasonal variation in the division rates of these ciliates, revealed in the original statistical analysis by Richards and Dawson,[5] is correlated with the variation in the amount of sunlight at different times of the year. The maximum of the division rate data occurs in July; likewise the maximum amount of sunlight in this region occurs in July (Fig. 1). This is not true in other localities. Wang[7] finds that

[6] Dawson, J. A., 1928, *J. Exp. Zoöl.*, LI., 199.

[7] Wang, C. C., 1928, *J. Morph. and Physiol.*, XLVI., 431.

there were more sunny days at Philadelphia during September and October, and further that there were more infusoria in the surface water of an open pond during these same months. His figures show that the number of infusoria is more closely related to the amount of sunshine than to the temperature of the water. I have superimposed his figures for temperature and for the number of infusoria, and have added the approximate amounts of

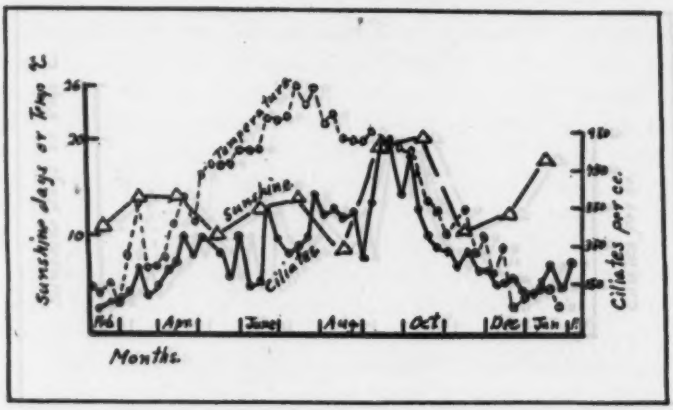

FIG. 3. Data from Wang.[7] The number of ciliates, temperature and amount of sunlight for the surface water of an open pond at Philadelphia. Note: Maximum number of organisms and sunlight in September and October. (*Cf.* Fig. 1).

sunshine in Fig. 3.[8] An inspection of the figure supports this conclusion.

Further corroborating evidence is to be found in another recent paper of Beers[9] who cultured *Didinium* under carefully controlled conditions and found no rhythms during a period of 265 days. The organisms were maintained in "diffused daylight" during the day time and in total darkness in an incubator at night. Dawson, however, kept his organisms in front of a window that received sunlight during a considerable part of the day protected

[8] Wang,[7] Chart 1, graph 1, and Chart 2, graph 3. He gives sunlight as days clear and partly cloudy. I have counted a partly cloudy day as about 55 per cent. of a clear day, which Dr. Brooks, meteorologist, Clark University, advises me may be less than the true value. The unsatisfactory histogram plot that Wang uses is avoided in Fig. 3. (*Cf.* Footnotes 3 and 6.)

[9] Beers, C. D., 1928, *J. Exp. Zoöl.*, LI., 485.

only by a drawn, light window curtain. Hence Dawson's data (used in the present study) would be expected to exhibit an effect of sunlight more clearly than would be expected with the more completely controlled environment used by Beers.

V.

No record of the temperature of Dawson's cultures is available. Since January and February, at Cambridge, are colder than the latter part of the year we might expect a lower rate of division early in the year, and this may account for the fact that the division rate is lower during the first quarter of the year than would be expected from the amount of sunshine at this time. This is also true of Wang's observations. The food supply of a pond is less in January and February than in the latter part of the year, which also explains part of this difference. Part of this deviation (Fig. 1) is due to the differences in amount of sunshine recorded for this part of the year for the different years. The drop in the composite division rate curve for June is due to the disturbance of moving the cultures to Woods Hole. They are again disturbed when they are returned to Cambridge. The effect of the return is not as obvious because the time of return varied from year to year while the opening of classes brought Dawson to Woods Hole at essentially the same time each year. During September 1925 and 1926 the cultures were cared for by an assistant during the absence of Dawson with a resulting abnormal drop of the division rate during this month.

Examination of the division rates of these organisms since the original analysis shows that the secular trend has continued until almost the end of 1928 when a new upward trend begins.[4] The data are too few to establish this new trend. The yearly cycle of variation is less pronounced the longer the cultures are maintained which suggests an accumulative effect of some unfavorable influence, or, of some deficiency, in the protoplasm of these organisms. Such an effect would be more obvious with unicellular than with multicellular organisms owing to the direct continuity of the protoplasm of the former. This trend has persisted despite gradual improvement and refinement of the technique for culturing. The relation between the trend and the diminution of

the magnitude of the cycle suggests that the loss of the ultra-violet or near ultra-violet rays may be the cause of the downward trend and diminished cycle of seasonal variation. The organisms are kept in glass moist chambers inside of a glass window so that a considerable part of the shorter wave lengths of light must be absorbed before reaching the animals. The influence of the shorter wave lengths of light on the division rate of protozoa could be determined by suitable experiment and should be evaluated in future studies made with these animals in more adequately controlled environments.

Summary.

1. Previous analysis of the division rates of *Paramecium aurelia* (mutant), *Blepharisma undulans*, and *Histrio complanatus* grown separately in pedigree isolation culture, under as nearly identical conditions as possible, for a period of 3 years, disclosed a secular trend and a seasonal rhythm for each organism. The seasonal rhythm has a maximum in July.

2. This seasonal rhythm is shown to be related to the amount of sunshine reaching the locality of the cultures. The maximum amount of sunshine is received in July also.

3. After the effect of trend and the influence of the amount of sunlight are removed from the division rates, they show no relation to each other except for deviations caused by known changes in the culture technique. Each organism has a division rate varying independently of the others, when the effect of external unifying influences are removed.

4. Consequently, the amount of sunlight, other conditions held constant, seems to determine the similarity of the division rate of these diverse organisms. The temperature is a secondary determining factor which has apparently less influence than sunlight when both variables are present in these experiments. Data from other investigations supports these conclusions.

5. It is suggested that the downward trend of the rates and the diminution of seasonal cycle which continue under laboratory conditions may be due to an accumulative deficiency of light of the shorter wave lengths which is absorbed by the containers, and that this effect be evaluated in studies made with more nearly constant environments.

THE EXCRETORY ORGANS OF TERRESTRIAL NEMERTEANS.

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In all except one of those species of terrestrial nemerteans which have been fully studied histologically the excretory system consists of numerous isolated nephridia, each of which leads to the surface of the body by a separate efferent duct. Such a system differs from that found in typical marine nemerteans mainly by the absence of the pair of longitudinal collecting tubules usually so conspicuous in the latter.

In a recent paper, dealing with the land nemerteans, Mary L. Hett ('27)has included an excellent comparative statement of the principal anatomical details which characterize the twelve known species of the genus *Geonemertes*. In regard to the nephridia of *G. agricola*, however, the brief description of this system given in my paper ('04) leaves some ambiguity as to the precise relations of terminal organs and efferent ducts. For this reason, and because further insight into the relationships of the different species of these aberrant land forms is highly desirable, this supplementary note on the nephridial system seems appropriate.

A recent study of well preserved material of sexually mature individuals of *G. agricola* collected near the shore of Hungry Bay, Bermuda, proves that the excretory system agrees rather closely with that described by Schroeder ('18) for *G. palaensis* and by Miss Hett ('24) for *G. hilli*. In all three species the system extends throughout the entire length of the body, with hundreds or thousands of isolated nephridia and the same number of efferent ducts.

Each of these numerous nephridia consists of a cluster of slender terminal organs (flame cells), with a comparatively thick-walled convoluted tubule and a slender efferent duct. Sections of the convoluted tubule, except for their smaller size, are similar to those of the longitudinal collecting tubules which join together all the

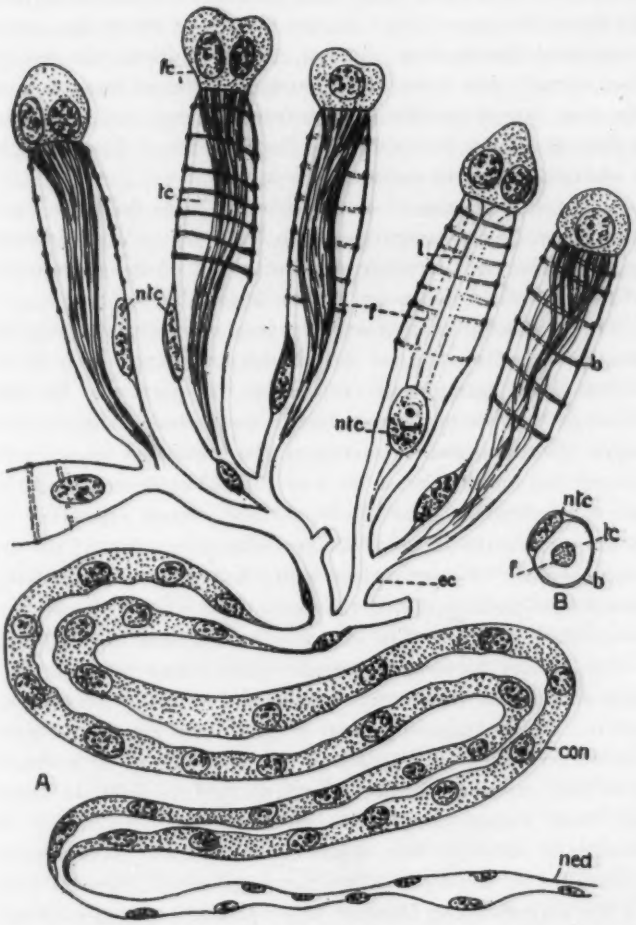

FIG. 1. *A*, Diagram of a single nephridium of *Geonemertes agricola*, showing five terminal organs connected with the convoluted tubule (*con*) which leads to the exterior of the body by the slender efferent duct (*ned*); *b*, thickened bar in wall of terminal chamber (*tc*); *ec*, end canal; *f*, flagellum arising from flame cell (*fc*); *l*, longitudinal bar; *ntc*, nucleus of terminal chamber. *B*, transverse section through a terminal organ. × 2500.

flame cells on each side of the body in the more typical nemerteans In my earlier paper ('04) I came to the conclusion that several convoluted tubules from adjacent clusters of flame cells were, in fact, actually thus united but I am now convinced that this is not the case, except, possibly, in rare instances, and that longitudinal collecting tubules do not occur. Each cluster of flame cells has a separate duct to the surface of the body.

I take this opportunity of describing in more detail the excretory system of *Geonemertes agricola*, the common land nemertean of Bermuda, and of comparing it with that of the other species of the genus in so far as our present knowledge will permit.

The most anterior nephrida are found in the head, about half way between the brain and the anterior extremity. They lie imbedded in the parenchyma and open to the exterior by the most direct path, either to the dorsal or to the ventral surface, according to their position. Posterior to the brain they occur in the parenchyma on all sides of the fore gut and proboscis sheath, but are most numerous near the dorsal and ventral aspects of the lateral nerve cords and near the ventrolateral margins of the proboscis sheath. In most regions of the body they are more abundant in the parenchyma on the ventral side of the lateral nerve cords than elsewhere. The total number is several hundred.

The number of terminal organs (flame cells) connected with each nephridium varies considerably, but is usually between six and ten. They frequently occur in pairs, each end canal supplying two terminal organs. Each of the latter consists of a slender, cylindrical terminal chamber from 0.015–0.024 mm. in length and from 0.0025–0.0038 mm. in diameter. Occasionally the chamber is distended to a width of 0.0045 mm. These dimensions differ but slightly from the figures given by Schroeder ('18) for the corresponding chamber in *G. palaensis* (0.014–0.02 mm. long and 0.004 mm. wide). Miss Hett ('24) finds very much smaller chambers in *G. hilli*, the average size in that species being only 0.008 by 0.0015 mm.

At the distal end of the chamber is a binucleated cell body of somewhat greater diameter than the chamber itself. Each of the two nuclei is about 0.002 mm. in diameter. It is quite possible that each nucleus represents a separate cell, but I have found no indication of a separating cell membrane.

The lash of cilia fills the greater part of the chamber (Fig. 1). Occasionally the preservation is such as to reveal the individual flagella of which the lash is composed, as indicated in one of the chambers shown in the figure.

The wall of the terminal chamber is extremely thin but is reinforced by a parallel series of six to eight narrow circular or spiral bars, or thickenings, situated at regular intervals (Fig. 1). A more delicate longitudinal bar is sometimes seen to extend about half the length of the chamber, starting at the base and joining the circular bands. Since this longitudinal bar is often placed symmetrically with regard to the two nuclei in the terminal cell body it is possible to conceive of the wall of the chamber as being formed of two symmetrical halves, joined together by the longitudinal bar. A third nucleus lies close against the wall of the chamber near its connection with the end canal (Fig. 1) and this presumably represents a cell which is actively concerned with the formation of the wall.

The series of circular bars extends through about half the length of the chamber, the most proximal bar lying not far removed from the nucleus near the proximal end of the chamber (Fig. 1). These relations are considerably different from those described and figured by Schroeder ('18) for *G. palaensis,* where the series of bars reaches only half way from terminal cell to the proximal nucleus. Schroeder also describes two longitudinal bars of considerable prominence.

A short, narrow and thin-walled end canal, with a branch to each of the flame cells of the cluster, leads directly into the distal end of the convoluted tubule (Fig. 1). The latter has relatively thick walls with granular cytoplasm and scattered nuclei, but is without cell boundaries. This portion of the nephridium also lies in the parenchyma and doubtless has an active excretory function.

The convoluted tubule is similar to the main longitudinal canal of typical nemerteans in its essential structure and its function is presumably identical. After making one or two loops in the parenchyma the walls become gradually thinner, leading to the slender efferent duct which passes through the body walls to the minute opening on the surface of the ciliated integument.

Comparison with Other Species.—In only five of the twelve

described species of terrestrial nemerteans have the nephridia been found but, as Miss Hett ('28) has pointed out, this fact should not be taken as indicating that they are not present in life. Such delicate structures are always difficult to find in improperly fixed material. All except one of these five species agree in having very numerous isolated clusters of flame cells, each group with its own efferent duct to the exterior and thus lacking the longitudinal collecting tubule which is frequently the only part of the system mentioned in anatomical descriptions of most littoral nemerteans. *G. chalicophora,* the natural habitat of which is unknown, is the only terrestrial form described as having a pair of such longitudinal tubules. Böhmig ('98) mentions ten pairs of efferent ducts for this species.

Schröder ('18) found that the excretory system in *G. palaensis* consists of many thousands of isolated nephridia, each with its own efferent duct. The number in the single specimen available for study was estimated by him to be about 35,000. Each nephridium is composed of several pear-shaped terminal organs, situated in the parenchyma internal to the body musculature, the extremely slender efferent duct passing with many convolutions through the muscular layers, cutis and epidermis to open at the outer surface of the ciliated cells on the lateral, latero-dorsal and latero-ventral aspects of the body. Usually ten or more pairs of terminal organs open into each tubule. The cytoplasm of the cells of the tubule contains granules and globules, but no cilia were found. In some sections the flame cells are so numerous as to make an almost continuous layer beneath the body musculature.

Only in minor details therefore does the system in that species differ from the conditions found in *G. agricola,* although the estimated number is vastly greater than in the latter species. In other anatomical features the two species differ widely.

The excretory organs of *G. hilli* have also been fully described and figured by Hett ('24). This species likewise has very numerous isolated nephridia of similar structure but of much smaller size. She has also ('28) found in *G. australiensis* organs of a similar type.

In no other nemerteans, so far as known, is the excretory system closely similar to that of the terrestrial species, the nearest approach, perhaps, being in the fresh water species of the genus

Prostoma. Here the terminal organs lead to a number of short and disconnected longitudinal canals. In species of *Cephalothrix* there are numerous isolated nephridia, as described by Wijnhoff ('10), but in those species the terminal organs are more nearly spherical and are multinucleate, with only a single terminal organ connected with each efferent duct.

All parts of the nephridium, except the efferent duct where it passes through the body wall, lie free in the parenchyma and hence are not in contact with the blood vessels. The fluid excretions of the terminal chamber as well as the substances excreted by the convoluted tubule must therefore be taken directly from the parenchyma. The latter is of a semifluid or gelatinous consistency and hence readily permeable by soluble materials brought to the vicinity by the blood vessels.

The blood-vascular system of the land nemerteans differs from that of most other groups in having numerous valve-like cells in the walls of the smaller vessels. In *G. agricola* the vessels are profusely branched in the anterior half of the body and the valve cells are very conspicuous. Similar cells are found in the vessels of some of the fresh-water nemerteans, and this is a further indication of the close relationships of the two groups.

LITERATURE.

Böhmig, L.

'98 Beiträge zur Anatomie und Histologie der Nemertinen. Zeits. wiss. Zoöl., 44, pp. 479–560.

Coe, W. R.

'04 The Anatomy and Development of the Terrestrial Nemertean (*Geonemertes agricola*) of Bermuda. Proc. Boston Soc. Nat. Hist., 31, pp. 531–569.

Hett, Mary L.

'24 On a New Land Nemertean from New South Wales. Proc. Zoöl. Soc. London, 1924, pp. 775–787.

'28 On Some Land Nemerteans from Upolu Island (Samoa), with Notes on the Genus *Geonemertes*. Proc. Zoöl. Soc. London, 1927, pp. 987–997.

Schröder, O.

'18 Beitr. zur Kenntniss von Geonemertes palaensis Semper. Abh. Senckenb. Nat. Ges., 35, pp. 155–175.

Wijnhoff, G.

'10 Die Gattung Cephalothrix und ihre Bedeutung für die Systematik der Nemertinen. Zoöl. Jahrb., Abt. Anat. u. Ont., 30, pp. 427–534.